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Dietary protein restriction in chronic renal failure

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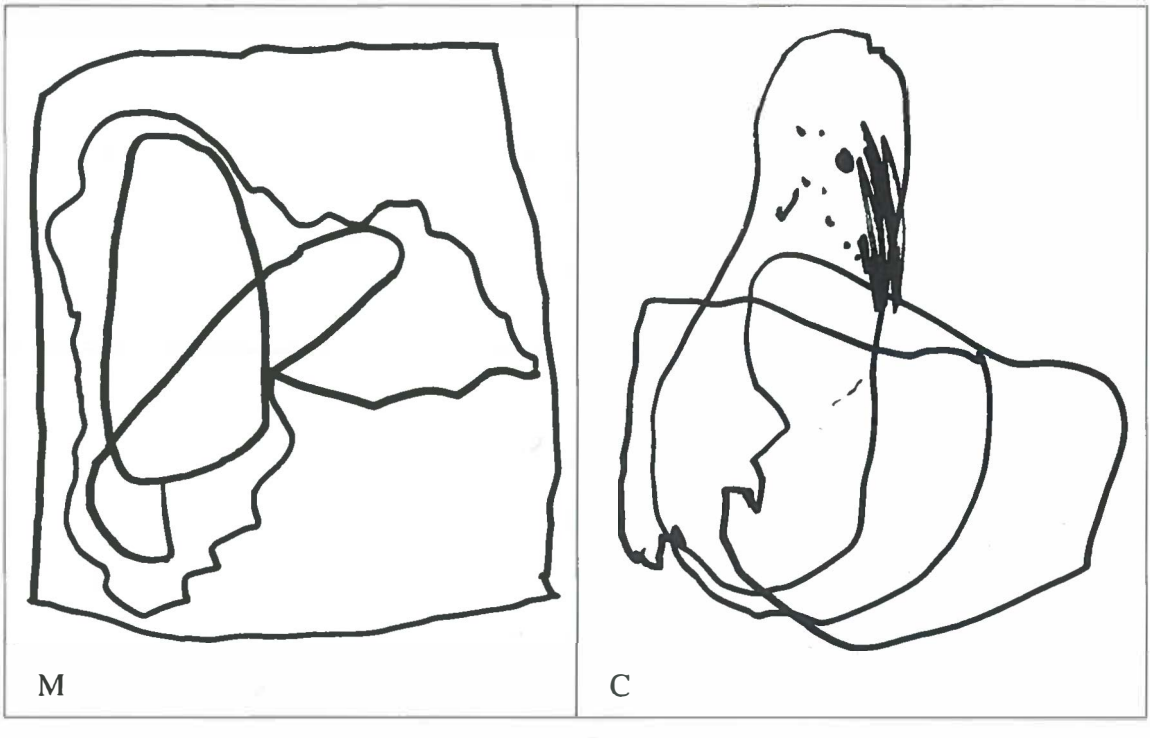
DIETARY PROTEIN RESTRICTION IN CHRONIC RENAL FAILURE

RESULTS FROM A PROSPECTIVE, RANDOMIZED TRIAL IN 247 PATIENTS



JOHAN ROSMAN

DIETARY PROTEIN RESTRICTION IN CHRONIC RENAL FAILURE

RESULTS FROM A PROSPECTIVE, RANDOMIZED TRIAL IN 247 PATIENTS

Stellingen

1. Het grootste probleem in de beoordeling van studies betreffende de invloed van interveniërende factoren op de nierfunctie, wordt gevormd door de statistiek.
2. Eiwitbeperkte diëten hebben een **beperkte qualitatieve** doelgroep, nl patiënten van het mannelijk geslacht met glomerulonefritis.
3. Eiwitbeperkte diëten hebben een **grote quantitatieve** doelgroep, nl patiënten van het mannelijk geslacht met glomerulonefritis.
4. Het beperken van de eiwittoevoer in het dicet met het doel de progressie van de nierinsufficiëntie te beïnvloeden is een fysiologischer maatregel dan het voorschrijven van een Angiotensine-I-converterend enzymremmer.
5. De eiwitbehoefte van een gezonde volwassene bedraagt 0.8 gram per kilogram lichaamsgewicht per dag.
6. De hyperfiltratietheorie speelt in de pathogenese van de progressie van nieraandoeningen slechts een bescheiden rol.
7. De belangrijkste factor voor het verkrijgen van dieetcompliantie is een goed samenspel in de driehoeksrelatie patiënt(e)-diëtist(e)-arts.
8. Internationale multicenter trials naar het effect van eiwitrestrictie hebben een zeer beperkte waarde, aangezien men het dieet van bijvoorbeeld een Italiaan niet met dat van een Skandinaviër kan vergelijken.
9. Het plotten van de reciproke serum creatininewaarden tegen de tijd om de progressie van een nierinsufficiëntie te beoordelen, is obsoleet.
10. Plasma measurements of intravenously administered ^{51}Cr-EDTA in order to calculate GFR is unreliable if renal function is severely impaired.
(Scand J Urol Nephrol 12: 133, 1978).
11. Patiënten met cystennieren zijn wat de snelheid van nierfunctieverlies aangaat, voornamelijk afhankelijk van een goede bloeddrukregulatie.
12. Wat iemand niet eet, dat deert hem niet.

13. Soms ziet men door de beeldschermen de dokters niet meer.

14. Niet om kunnen gaan met een personal computer is een moderne vorm van analfabetisme.

15. De arrogantie van de Nederlandse vakantieganger in het buitenland laat zich mathematisch beschrijven met de formule $A=PxDV$. Hierbij staat A voor de mate van Arrogantie, P voor de Plek op Aarde en DV voor de Duur van het Verblijf.
(Met toestemming overgenomen uit: 'Meest Modernismen' door Kees van Kooten, Uitgeverij De Bezige Bij, Amsterdam, 1989).

16. Een echte vent strijkt zijn eigen overhemd.

17. De Zwitserse Horloge-industrie, altijd fervent tegenstander van elektronische horloges, profiteert van het feit dat de moderne mens omschakelt van high-tech naar high-mech levensstijl.

(Stellingen behorend bij het proefschrift van Johan Rosman, 30 mei 1990.)

Rijksuniversiteit Groningen

DIETARY PROTEIN RESTRICTION IN CHRONIC RENAL FAILURE

RESULTS FROM A PROSPECTIVE, RANDOMIZED TRIAL IN 247 PATIENTS

Proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels,
in het openbaar te verdedigen op woensdag 30 mei 1990
des namiddags te 2.45 precies

door

Johan Boudewijn Rosman

geboren op 2 december 1956
te Groningen.

Promotores:	Prof. dr. G.K. van der Hem Prof. dr. A.J.M. Donker
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To Merith and Chris

To the cover:

These drawings are spontaneous expressions of children of 3, resp. 6 years old, if only the word 'kidney' is given to them.
This noun means nothing to them; but they have their imagination and are able to produce some kind of picture.
It is comparative to our knowledge of the kidney. Although we know what this organ looks like, the understanding of its way of functioning is not much more than a child's knowledge of the outer appearance.

(Drawings kindly reproduced by permission of Merith and Chris Rosman)

Contents

Acknowledgements

Sitting behind screen and keyboard, thinking of all people who made this study and my promotion possible, my first thoughts concern Truus Piers-Becht, who as our dedicated dietician conducted the paramedic part of this ambitious trial. It was a strike to all of us, when we heard of her much too early sudden death in March, 1988. As one of the initiators of the study, her delightful way of working is credited by the excellent dietary compliance achieved.

The innovator of the study is undoubtedly Ab Donker. The combination of the marvellous quality of his grey cells and the stimulating way of discussing the results, made this trial to what it became.

Prof.G.K. van der Hem, Head of the Department of Nephrology and promotor, gave me the opportunity to conduct this trial in his outpatient clinic, with its excellent infrastructure. He also arranged many things with respect to the promotion and without his help, the 30th of May would have been an ordinary working day for me.

Mathematical brains behind the study was Wim Sluiter. In Holland we would call him 'Willie Wortel', after a famous comic strip-figure, and I can call myself lucky to be his 'Lampje' from time to time.

Psychological support in stressful times was supplied by Marry, who took care of our children the many hours I was hidden in the University Computing Center. Although we decided to go our own ways, I'll never forget her positive life-vision, stimulating me to go on.

The cooperation with Piet ter Wee, my nearest collegue in Groningen, gave rise to the often heard expression: 'Piet en Johan, twee handen op één buik'.

Living in Germany, and now in Switzerland, Uli helped me in taking distance from the work from time to time. I know this is a hard job with A-type people, but she managed to make me realize that during weekends there are also other things in life as computing.

I am much indebted to my fellow members of the 'European Study Group for the Conservative Management of Chronic Renal Failure'.We became a close group of friends over the last years. In this respect I want to mention specifically:
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The constructive discussions we had at our meetings formed an ongoing basis for this work.

My medical and scientific career was supported by many people. In first instance of course by my parents, who made it possible for me to study medicine.

Then, in chronological order: Prof. dr. H.J. Sluiter, who awoke my scientific interest in 1978. Prof. drs. H. Scholten and Prof. dr. E. Mandema who gave me the necessary training in Internal Medicine, later followed by Prof. dr. W.D. Reitsma.

Prof. Arthur Teuscher from the Department of Diabetic Medicine at the University of Bern, Switzerland, trusted me to work on his large projekt on diabetic risk factors in autumn/winter 1984. I am very happy that the warm friendship with him lasts.

In October 1987, I went to Heidelberg, FRG, where Prof. E. Ritz, Prof. J. Bommer and Prof. K. Andrassy further enabled my internal medicine training in the direction of Nephrology.

In May, 1989, Prof. P.Weidmann gave me the opportunity to work again in Switzerland. His stimulating and engaged way of performing science is impressive and for me a passport to be scientifically productive in the coming years.

In March 1990 I changed again, now to Basel, where Prof. G. Thiel and Prof. F. Brunner further accompany me on my way through the fascinating world of nephrology.

This thesis could not have been written without the background-activity of so many people, from most of them I do not even know their names.

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I thank all those who take this book at hand and are willing to read it. Even if they don't make it from page 1 to the end. I am not disappointed if they use it to fill their bookshelf; I know my own bookshelf.

Chapter 1: Introduction

Dietary protein restriction and the progression of renal failure

In the last two decades there has been a growing interest in the use of protein-restricted diets to prevent or retard the progression of renal failure. The underlying mechanism by which such a diet is able to counteract progression has been intensively studied in the so-called remnant kidney model where rats undergo 5/6 nephrectomy. Micropuncture studies were employed to reveal the hemodynamic effect on a glomerular level.

The functional adaptation to nephron loss appears to be an increase of single nephron glomerular filtration rate (SNGFR) and of two of its determinants: glomerular plasma flow rate (Q_a) and intra-capillary pressure in the glomerulus($\bar{P}_{gc}$)[1]. The SNGFR increments are to a certain extent inversely proportional to the number of surviving nephrons. The increased transcapillary flux leads in due course to a desintegration of the glomerular capillary wall, which becomes protein-permeable. Structural changes two weeks after renal ablation consist of attenuation of glomerular epithelial cells with an increase in mesangial cells and matrix, well-known precursors of glomerulosclerosis, the ultimate cause of end-stage renal failure in this model[2].

In the remnant kidney model the increase in $\bar{P}_{gc}$ (glomerular hypertension) seems to be the main factor responsible for the development of progressive glomerulosclerosis, clinically obvious as proteinuria. In the human situation this hypothesis is supported by a study, that showed that proteinuria diminished in a patient with polycythemia (increased viscosity raises $\bar{P}_{gc}$) after phlebotomy[3]. In the animal experiment, lowering of $\bar{P}_{gc}$ in 5/6 nephrectomized rats by means of enalapril (an angiotensin-1 converting enzyme inhibitor: CEI), was accompanied by a significant reduction in proteinuria, compared to control animals not receiving enalapril[4]. Hemodynamically an increase in Q_a with unaltered SNGFR was noted, so the filtration fraction (FF), as defined by the quotient of GFR and effective renal plasma flow (ERPF) dropped. Comparable results were obtained by treating insulin dependent diabetic rats with with CEI. High SNGFR, accompanied by high FF in the early stage of this disorder normalized after giving enalapril, apparently by lowering the $\bar{P}_{gc}$. Strikingly, these animals did not develop the expected proteinuria[5].

The use of protein-restricted food in the remnant kidney model is known to reduce $\bar{P}_{gc}$ and glomerular protein loss and to prevent the development of progressive glomerulosclerosis.

What, then, might be the mechanism by which protein restriction lowers $\bar{P}_{gc}$?

In normal individuals several pathways are known to increase GFR (see figure). One of these is the intravenous administration of a low dose of dopamine, which has no effect on blood pressure or heart rate. Infusion of dopamine results in increased Q_a, a rise in GFR and a decrease in FF[6]. Another way is the ingestion of a meat meal or an amino-acid

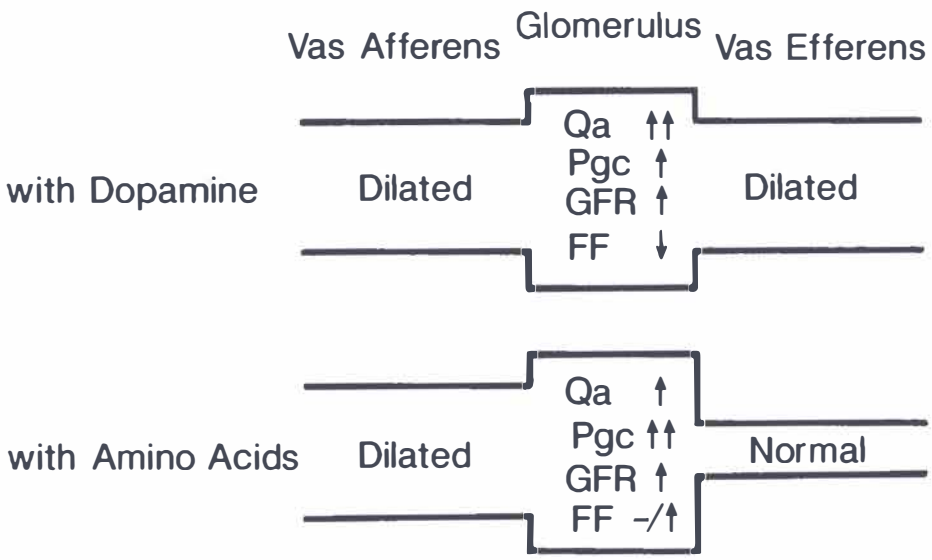

Figure 1 Renal hemodynamics in healthy subjects.

infusion. Now the increase in GFR is accompanied by a slight increase in the FF. Ter Wee et al concluded that exogenously administered dopamine leads to an increase in Q_a by means of afferent *and* efferent vasodilation, whereas a meat meal or amino-acid infusion leads to a rise in $\bar{P}_{gc}$ by predominant dilation of the afferent arterioles[7]. When the same experiments are carried out in patients with a GFR below 50 ml/min/1.73 m^2, the results are different. Then dopamine infusion is followed by only a slight increase in ERPF and neither method appears to be able to increase GFR. Obviously, in those patients there can be no further increase in GFR because remnant nephron function is already maximal, or so to say, the renal functional reserve (RFR) capacity is exhausted by means of maximal hyperperfusion/-filtration.

The RFR-capacity hypothesis was introduced by Bosch et al[41] and defined as the difference between stimulated and unstimulated GFR. This concept was recently in extenso reviewed by Rodriguez-Iturbe[137].

Data from micropuncture studies of rat kidneys confirm the above mentioned impression. The glomerular blood flow is mainly a function of the afferent and efferent vascular resistance. This 'renal autoregulation-system' is disturbed in case of reduced functional renal mass or certain diseases, like diabetes mellitus. The above mentioned increases in Q_a and SNGFR are related to changes in the balance afferent/efferent vascular resistance.

There is strong evidence that hyperfiltration due to a meat meal or amino-acid infusion is induced by a circulating hormonal factor, since in isolated perfused kidneys, GFR does not increase after amino acid infusion[138]. Several mediators are under discussion. One of the candidates is glucagon[42], since it is known to be released from the pancreas after amino acid infusion and glucagon infusion increases GFR and ERPF. In normal individuals a meat meal after blocking the glucagon response by means of a priming dose

of somatostatin is not followed by an increase in GFR[9]. However, somatostatin has a wide spectrum of inhibitory actions and the postprandial increase of GFR precedes the increment in plasma glucagon levels[140]. Moreover, infusion of glucagon into the renal artery of dogs does not change GFR whereas infusion of glucagon into the portal vein, results in a significant increase in GFR[44,141]. So, the existence of a liver-dependent compound was discussed[12]. One of them is glomerulopressin, a hormone first found in the toad by Uranga[44,45]. Alvestrand et al[12] postulated that this hormone, that hypothetically causes a reduction in the afferent vascular tone (thus increasing $\bar{P}_{gc}$), is secreted by the liver. However, data from Woods et al question this theory, since similar GFR-increments could be obtained in dogs with ligated liver vessels[139].

Kleinman et al found that in patients with a deficiency of growth hormone (GH), GFR is in the low range of normal, while on the other hand, these patients do not show a response to a protein load[10]. Nor does protein loading increase GFR in patients with IDDM[11]. It is well-known that in diabetics GH-levels are high and GFR is increased. Individuals with IDDM and supranormal GFR, however, show the usual response to infusion of dopamine[129]. So, diabetic patients may have an increased GFR due to afferent vasodilation and a resulting increase in $\bar{P}_{gc}$, a hypothesis being in accordance with the high normal FF in patients with IDDM.

Certainly it is not GH itself, that is responsible for the observed GFR increase, since this increment starts after a delay of a few days if healthy volunteers are treated with GH[14]. The effect also persists some days after stopping GH treatment, which gave rise to the idea that the GH-induced release of Insulin-like Growth Factor-1 (IGF-1) may be the responsible component[13,14,130]. That the augmented GFR matches with IGF-1 levels could in the meantime be confirmed[14].

Coming now to the possible clinical relevance of these findings, one may ask whether a low protein diet is capable of reversing the disbalance between afferent and efferent tone and reduce $\bar{P}_{gc}$, thus protecting the glomerular capillary wall. If it is effective, would it be beneficial in all forms of renal disease[15], and if so, at what stage of renal failure should it be started? In general, several studies, retrospective as well as prospective ones, have confirmed the benefit[16,17], but not all questions have been answered yet[18].

The only definite proof can come from prospective trials in sufficiently large groups of patients and to meet this challenging concept was the aim of this thesis.

Aims of this study

Knowing that protein-restricted diets had shown beneficial effects in retrospective studies, mostly with only limited numbers of patients, we decided in 1982 to start a large prospective, randomized trial.

The trial had several goals:

1) does a low protein diet really retard the progression rate of chronic renal disease ?

2) if so, does this hold true for all diagnosis groups ?

3) are patients compliant to low protein diets ?

4) do low protein diets have considerable side-effects like malnutrition, negative psychosocial repercussions or unfavourable shifts in amino-acid composition ?

Between 1982 and 1984, 247 patients could be included in the trial. They all were recruited in the nephrology-outpatient clinic of the University Hospital of Groningen. Its unique infrastructure gave us the opportunity to study this large number of patients for over four years in a one center trial.

A one center trial has tremendous advantages over multicenter trials, where problems may arise concerning comparability of data between centers. For example, in Groningen all patients were seen by the same dietician throughout the entire study, which warranted a comparable composition of the diet between the patients and a homogeneity in data.

Chapter 2 of this thesis contains detailed information concerning patients and methods of the study and presents the data obtained after a follow-up of two years. The results were generally positive.

Chapter 3 is a more extensive analysis of the response to the diet in the various diagnosis groups and discusses the statistical problems encountered in the study.

Chapter 4 further explores statistics and it is concluded that the reciprocal value of serum creatinine to monitor renal function over time, as promoted by Mitch and Walser in 1974 and used in Chapter 1, is not reliable enough to draw definitive conclusions.

Chapter 5 describes the influence of dietary protein restriction on proteinuria and a relationship between initial protein excretion and responsiveness to the diet is discussed.

Chapter 6 presents the most recent data on dietary protein restriction available. Results of four years follow-up are given and important conclusions regarding the identification of the target group are drawn. Furthermore, the first results of an investigation of amino-acid profiles in patients with chronic renal failure, with and without protein restriction are presented.

Chapter 7 specifically analyses in more detail the amino-acid profiles and their relation to nutritional status and progression of renal insufficiency.

Chapter 8, finally, discusses the impact of gender on the progression rate in our patient group and confirms data, derived from animal experiments, that male subjects show a more rapid decline in renal function but have a better response to the diet as compared to females, where no effect could be established at all.

In general, the initial optimism of the dietary effects on renal function in case of kidney failure is no longer justified. However, for a well-defined subset of patients, especially those with chronic glomerulonephritis, it remains a first-line therapy.

Chapter 2

Prospective randomised trial of early dietary protein restriction in chronic renal failure

Summary

In a prospective randomised study of 228 patients with various renal diseases, early moderate dietary protein restriction retarded the development of end-stage renal failure. 149 patients were followed up for at least 18 months; the protein-restricted patients showed a fall in serum urea and phosphate concentrations and in the 24 h excretion of urea, phosphate and protein. Regression analysis of the reciprocals of serum creatinine against time showed that the average rate of decrease in reciprocal creatinine was three to five times lower in the protein-restricted groups than in the control groups. These results confirm that moderate dietary protein restriction is an acceptable and effective way of delaying functional renal deterioration. The finding has implications for the management of chronic renal insufficiency.

Introduction

A protective effect of a low protein intake on the course of nephrotoxic serum nephritis was first demonstrated in 1939[19] and has since been confirmed[20]. There is convincing experimental evidence that restriction of dietary protein is effective in preventing functional deterioration in rats with reduced renal mass[1,21-23]; protein restriction prevents both hyperfiltration and histological lesions in the remnant glomeruli[1].

Does early restriction of dietary protein retard the progression of renal damage in man? Levin and Cade[24] found that in patients with chronic renal failure protein restriction not only alleviated uremic symptoms but also improved renal function. Kluthe et al[25] agreed that a low-protein diet slows down the progression of chronic renal failure in man, as is also shown in the majority of other studies of this question[14,26-34]. Yet, no prospective randomised trial of early protein restriction in man has been done. We started such a study in 1982 and report here its first results.

Patients and Methods

The subjects were patients of our nephrology outpatient department who visited the clinic between Jan 1, 1982, and April 1, 1984. To enter the trial patients had to have creatinine

clearances of 60 ml/min/1.73 m^2 or less and no lethal disease. Patients with systemic lupus erythematodes, polyarteritis nodosa and active Wegener's granulomatosis were excluded.

The 228 patients were stratified for sex, age and renal function and then randomly allocated to a protein-restricted (B or C) or a control (A1 or A2) group. Thus, sixteen groups were formed (fig 1). Table I gives the distribution of patients'renal diseases over the protein-restricted and control groups (X^2_{17}=13.4; not significant).

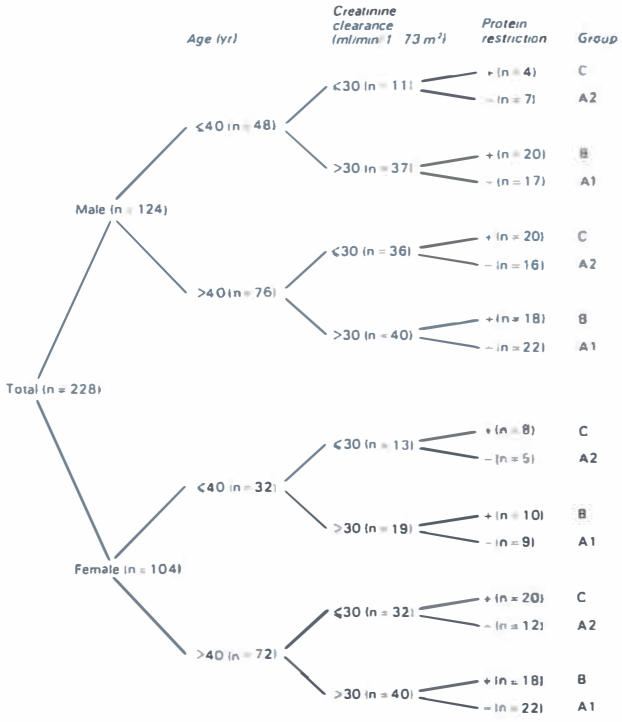

Figure 1 Distribution of patients after stratification for sex, age, and renal function.

All patients then visited the dietician and a diet history was taken. Patients allocated to the protein-restricted groups were asked to adhere to a low protein diet after consent was obtained; patients with creatinine clearance of 10-30 ml/min/1.73 m^2 were asked eat 0.4 g protein/kg body weight daily (group C), and patients with creatinine clearance of 31-60 ml/min/1.73 m^2 0.6 g protein/kg body weight daily (group B). Patients allocated to the control groups continued their usual diet. All patients received a supplementary vitamin/trace element preparation ('Dagravit totaal 30', Dagra) from the time of randomisation. The preparation contained 10 µg vitamin D_3. Patients allocated to the low protein groups received supplementary methionine if the calculations by the dietician showed a deficiency.

Table I Distribution of renal diseases over protein-restricted and control groups.

	Diagnosis	No in group				% of total
		A1	B	A2	C	
1.	Unknown	2	3	0	2	3.1
2.	Membranous glomerulopathy	5	4	1	3	5.7
3.	Membranoproliferative glomerulonephritis	1	2	1	1	2.2
4.	Focal glomerulosclerosis	7	12	6	5	13.2
5.	IgA glomerulopathy	5	3	1	0	3.9
6.	Glomerulonephritis (other types)	6	10	1	2	8.3
7.	Adult polycystic kidney disease	1	5	3	5	6.1
8.	Unilateral agenesis	2	1	1	1	2.2
9.	Unilateral nephrectomy	6	3	0	4	5.7
10.	Hypoplasia/dysplasia	1	1	0	1	1.3
11.	Pyelonephritis	3	2	4	3	5.3
12.	Alport's syndrome	2	1	0	0	1.3
13.	Nephrosclerosis	10	8	5	3	11.4
14.	Analgesic nephropathy	4	3	10	7	10.5
15.	Interstitial nephritis	1	0	2	3	2.6
16.	Reflux nephropathy	5	1	2	4	5.3
17.	Other urological disorders	4	1	2	3	4.4
18	Miscellaneous*	5	6	1	5	7.5

* Amyloidosis, diabetes, etc.

For all patients the objectives of diastolic blood pressure ≤ 90 mmHg, serum phosphate ≤ 1.65 mmol/l and serum calcium between 2.35 and 2.65 mmol/l were pursued. Diuretics, beta blockers and vasodilators, such as hydralazine and prazosin, were used to control blood pressure, and aluminium hydroxide for serum phosphate control if necessary. If serum calcium was below 2.30 mmol/l, dihydrotachysterol was given.

At randomisation, and thereafter every 3 months, the following variables were measured: body weight; sitting blood pressure measured on the right arm with a conventional sphygmomanometer; hemoglobin, hematocrit; creatinine, urea, phosphate, calcium, alkaline phosphatase, total protein, albumin, cholesterol, triglycerides; venous pH and bicarbonate. Every 3 months the 24 h urinary excretion of sodium, urea, creatinine, calcium, phosphate and protein was measured. For all determinations standard hospital techniques were used.

Every 3 months patients on a protein-restricted diet visited the dietician, who was informed about the 24 h urea excretion at the previous clinic visit. The diet was adjusted if necessary or preferred by the patient. The subjective acceptance of the diet by the patients was scored as 'fair' or ' bad'.

Patients in the control groups (A1 and A2) were advised to reduce their protein intake during the study if serum urea exceeded 25 mmol/l. In some patients renal function became severely impaired (creatinine clearance < 10 ml/min/1.73 m^2); they received cadaveric renal transplants as DR-identical kidneys became available. Other patients underwent haemodialysis when creatinine clearance dropped below 4 ml/min/1.73 m^2.

A data base for the variables was set up. Calculations were done on a CDC Cyber 170/760, partly using standard tests from the statistical package for the social sciences[35].

Statistical Analysis

When the study was designed, about 150 patients were known to be available for the trial. With this number of patients a 'survival' difference of approximately 25% is needed to be detected at p=0.05 with a 85% chance. Taking a persistent increase in the serum creatinine concentration of 25% of the level at entry as the non survival criterion, such a survival difference was expected to be reached after 18 months in view of the results of Maschio et al[29].

Since all known patients were gradually entered into the trial in the 9 months from Jan 1, 1982, the study was due to be terminated on April 1, 1984. New patients also entered the study; their data were analysed when follow-up was 3 months or longer.

Differences in deterioration of renal function were studied by log-rank analysis of survival[36] with persistent increases in the serum creatinine level of 5, 10, 20, 30, 40, 50, 75, and 100% as the non-survival criterion. Thus, for example, if a patient had a follow-up of 12 months and the increase in serum creatinine was 10% at 9 months but less than 10% at 12 months, the patient was regarded as a survivor when a non-survival cut-off of 10% was used. Log-rank analysis of trend on survival was done with a non-survival criterion of 10% increase in the serum creatinine concentration. Because of the difference in dietary advice, the survival curves of group B and C were analysed separately.

Differences in frequency distributions were tested by chi-square analysis, corrected for continuity. For comparison of the measured data between the groups, Mann-Whitney tests were used. Changes in variables within the groups were determined with the Wilcoxon matched-pairs signed-ranks test. The decline of the median reciprocal of serum creatinine was studied by linear regression analysis. For all statistical tests the level of significance chosen was two-tailed p=0.05.

Results

228 patients entered the study before Jan 1, 1984, and thus were followed up for at least 3 months. Median age at entry to the trial of the whole patient group was 47.8 years (range 15-73). The median ages of men and women were 48.5 years and 47.7 years (range 15-73 in both). The median ages of the younger age groups (≤ 40 years) were 31.9

years in men and 27.5 years in women, and those for the older age groups (>40 years) 57.2 and 52.5 years in men and women, respectively.

Log-rank analysis of trend revealed no influence of sex on renal insufficiency, although there appeared to be distribution differences in diagnoses between the sexes (Table II; $X^2_5=20.54$, $p<0.001$). Age, however, did have an influence: patients younger than 41 years showed faster progression ($p<0.05$). In the younger group there were more cases of glomerulonephritis and reduced renal mass, and fewer of interstitial nephritis and nephrosclerosis (table II; $X^2_5=18.01$; $p<0.005$). Because the patients were stratified for age and no significant differences in trend were established within the two age groups, separate statistical analysis of the age groups was not necessary.

Table III gives the numbers of patients who were removed from the study before Jan 1, 1984.

Table II Distribution of diagnoses between sexes and according to age.

	No (%)			
	Sex		Age (yr)	
	M	F	≤40	>40
Glomerulonephritis (2-6)	44 (35)	32 (31)	32 (40)	44(30)
Polysystic disease (7)	9 (7)	5 (5)	5(6)	9(6)
Reduced renal mass (8-10, 16)	18 (15)	15 (14)	18 (23)	15 (10)
Interstitial nephritis (11, 14, 15)	10 (8)	32 (31)	6 (7)	36 (24)
Nephrosclerosis (13)	20 (16)	6 (6)	4 (5)	22 (15)
Rest (1, 12, 17, 18)	23 (19)	14 (13)	15 (19)	22 (15)

* Nos in parentheses = diagnosis groups in table I.

Table III Patients removed from study.

	A1	A2	B	C
Died*	3	1	1	1
Dialysis	1	10	0	3
Transplantation	0	0	0	3
Lost	0	0	0	0
Total	4	11	1	7

* 2 patients in group A1 and 1 in group A2 died of end-stage renal failure having refused dialysis. 2 other patients died of myocardial infarction, and 1 of a cerebrovascular accident.

Log-rank analysis revealed a lower rate of increase in serum creatinine in the protein-restricted groups. As an example, fig. 2 shows the survival in the protein-restricted and control groups tested at a non-survival criterion of a persistent 10% increase in serum creatinine.

Table IV gives, for all chosen criteria of non-survival, the differences in survival at 24 months and the time after which the difference was first significant.

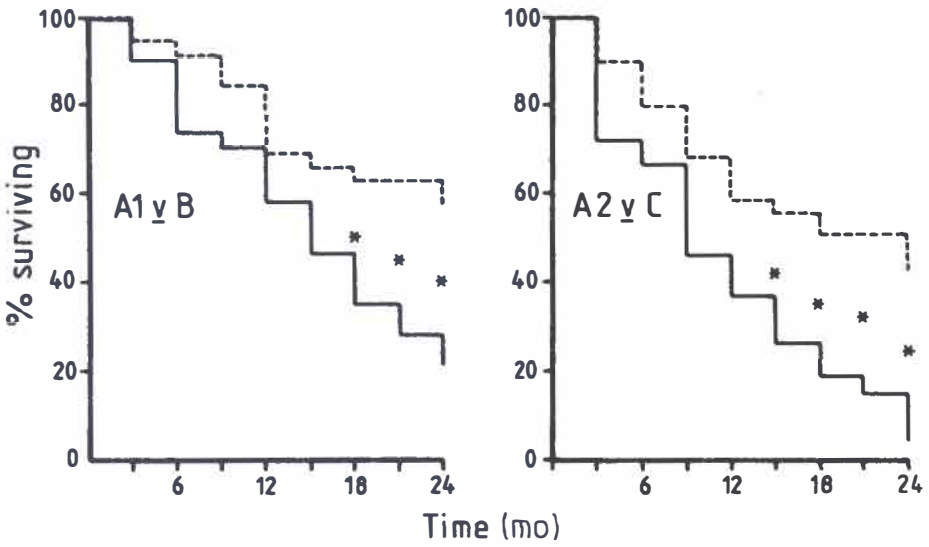

Figure 2 Survival curves for group A1 versus group B and group A2 versus group C with a persistent 10% increase in serum creatinine concentrations as non-survival criterion.

Broken lines = protein-restricted groups; solid lines = control groups. *p<0.01.

149 patients entered the trial before Oct 1, 1982, and thus could be followed up for at least 18 months. Over this time 14 patients progressed to end-stage renal failure requiring dialysis (Table III). Three patients underwent transplantation with a cadaveric kidney. When the dialysis-dependent or transplanted patients were still alive at April 1, 1984, their last

Table IV Survival at 24 months and time at which difference between groups became significant.

Non-survival criterion	Survival%		Time at significant difference (mo)	Survival%		Time at significant difference (mo)	Combined probability of survival difference
	A1	B		A2	C		
5%	13.2	48.5†	15	6.4	24.0	..	<0.001
10%	20.7	56.9†	18	5.3	42.9†	15	<0.0005
20%	21.8	66.4*	24	16.9	60.0‡	6	<0.0005
30%	42.3	65.2	..	36.1	73.9‡	6	<0.005
40%	..	..	..	42.0	72.3‡	12	..
50%	70.5	73.1	..	50.3	70.8*	12	NS
75%	..	..	..	56.1	79.6*	18	..
100%	..	..	..	69.5	98.0*	18	..

* p<0.05 † p<0.01 ‡ p<0.001.

serum creatinine value before dialysis or transplantation was used in the 3-monthly analyses thereafter. Fig. 3 gives the reciprocals of median serum creatinine values against time for the four groups. Protein restriction reduced the median rate of progression of renal insufficiency by a factor of 3 (group C) to 5 (group B).

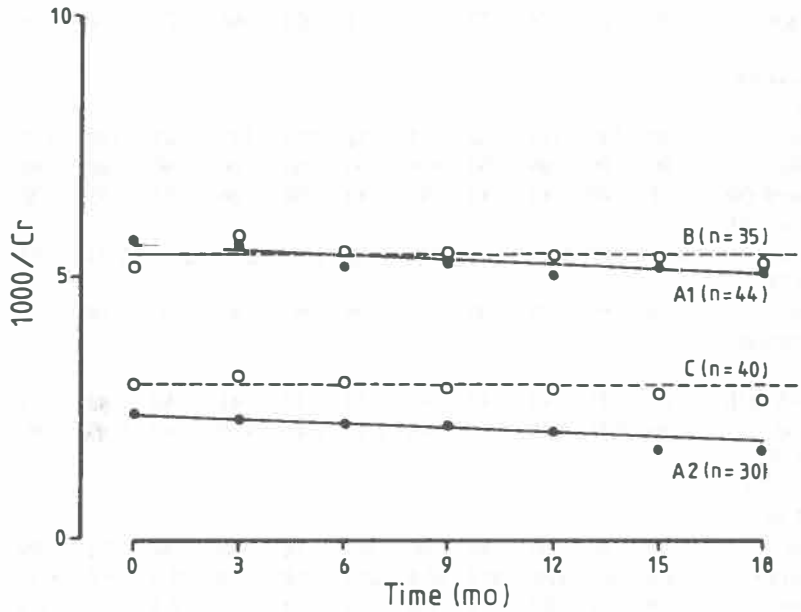

Figure 3 Relation of reciprocal of median serum creatinine (1000/Cr) concentration with time (in mo) in patients followed up for at least 18 months (n=149).

A1: 1000/Cr = 5.632 - 0.036 x time; r = -0.83, $p<0.05$.
A2: 1000/Cr = 2.430 - 0.037 x time; r = -0.93, $p<0.01$.
B: 1000/Cr = 5.437 - 0.007 x time; r = -0.26, not significant.
C: 1000/Cr = 2.910 - 0.016 x time; r = -0.72, not significant.

Reciprocal of serum creatinine was studied by linear regression analysis. For all statistical tests the level of significance chosen was two-tailed $p=0.05$.

No persistently significant differences in body weight, blood pressure, hematocrit, serum levels of calcium, alkaline phosphatase, albumin, or uric acid, or 24 h excretion of sodium and calcium were found within the protein-restricted and the control groups after 9 and 18 months' follow-up (Table V). In group C, however, 24 h excretion of creatinine was significantly lower than at entry after 9 and 18 months ($p<0.01$).

The effects of protein restriction on serum urea and 24 h urea excretion are shown in figs. 4 and 5.

Table V Median values of variables measured at entry and after 9 mo and 18 mo follow up.

	A1			B			A2			C		
	0	9	18	0	9	18	0	9	18	0	9	18
Body weight (kg)	70	70	73*	72	73	71	69	68	71	70	69	69
Blood pressure (mm Hg)												
Systolic	140	140	141	140	134	131	135	135	143	140	140	140
Diastolic	90	90	90	90	85*	90	90	90	96	90	90	84
Haematocrit (%)	41	40	41	42	42	42	38	36	37	37	36	36
Serum levels of:												
Calcium (mmol/l)	2.44	2.42	2.43	2.40	2.42	2.42	2.36	2.35	2.38	2.41	2.44	2.43
Alkaline phosphatase (IU/l)	94	90	89	99	97	98	81	88	76	88	79	79
Albumin (g/l)	42	43	43	42	43	42	42	41	42	41	41	43
Uric acid (mmol/l)	0.46	0.45	0.43	0.46	0.45	0.45	0.47	0.50	0.47	0.47	0.45	0.46
24 h excretion (mmol/l) of:												
Sodium	88	90	107	92	86	107	76	83	84	101	99	96
Creatinine	11.0	10.6	12.6	10.4	10.4	10.6	9.8	9.4	9.3	9.6	9.2†	8.6†
Calcium	0.8	0.7	0.8	1.1	1.0	1.4	1.3	1.1	1.4	1.1	1.3	1.2

*$p<0.05$ † $p<0.01$ for differences with time within groups.

Serum phosphate and 24 h phosphate excretion were significantly lower in the protein-restricted groups than in the control groups at 9 and 18 months of follow-up.

The effect of protein restriction on 24 h protein excretion was also studied in patients who had more than 0.5 g/24 h urinary protein loss at entry. In the control groups there was no significant change in proteinuria. In the protein-restricted groups, however, we found a significant reduction in proteinuria even after 3 months. At 9 months the p values were 0.02 for group B and 0.008 for group C.

At 3 and 6 months' follow-up the ratio between 'fair' and 'bad' acceptance of the diet was roughly 2/1. At 18 months this ratio was 7/1.

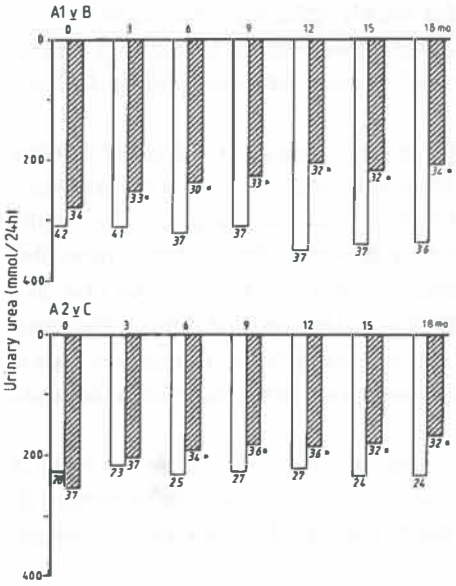

Figure 4 Median serum urea of patients followed up for 18 months.

Solid lines = control groups; broken lines = protein-restricted groups. Numbers in parentheses = numbers of patients for whom data available. *p<0.05 for differences between protein-restricted and control groups.

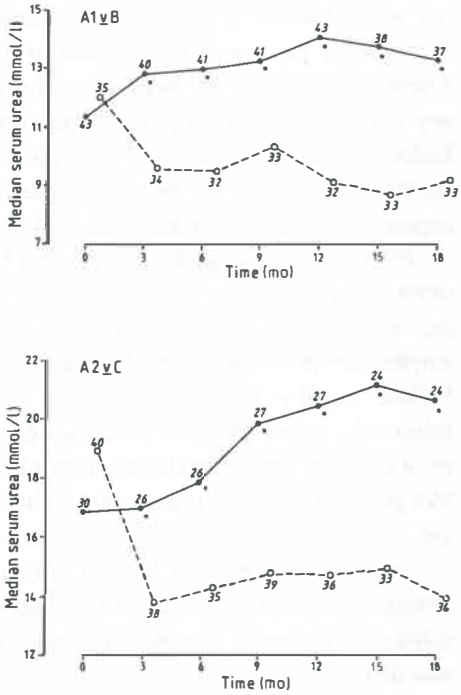

Figure 5 Median 24 h urinary urea of patients followed for 18 months.

Open bars = control groups; shaded bars = protein-restricted groups. Numbers at ends of bars = numbers of patients for whom data available. *p<0.05 for differences between protein-restricted and control groups.

Discussion

In this prospective randomised study we have shown that if patients adhere to a diet containing 0.4 - 0.6 g protein/kg body weight the rate of progression of chronic renal insufficiency is reduced. We did not yet perform our calculations in separate subsets according to diagnosis groups. Despite the generalisation, the outcome confirms previous retrospective and prospective non-randomised trials[26-34,37,38].

A diet containing 0.4-0.6 g protein/kg body weight appears not to be harmful provided that foods with protein of high biological value, such as potatoes, rice and eggs, are eaten and that the diet is isocaloric with the previous diet[39]. 0.5 g protein/kg body weight is recommended by the Food and Agricultural Organisation as a safe minimum protein intake for healthy adults[40]. No significant weight losses or significant falls in serum albu-

min were observed in our protein-restricted groups. The significant fall in 24 h creatinine excretion, a parameter for the lean body mass, in group C was already present at 3 months' follow-up and might be related to the fact that patients in this group ate hardly any meat. In normal subjects a vegetarian diet greatly reduces creatinine excretion[41]. Reduced creatinine production in group C, however, cannot explain the survival difference, for instance, with the 50% level as non-survival criterion. Moreover, such a difference appeared only after 12 months (table IV).

Protein-restricted patients were often hungry at first. Frequent visits to the dietician, often leading to adjustments of the diet, were able to solve this difficulty without affecting the excretion of urea or phosphate and thus the advised protein restriction. This finding emphasizes the importance of frequent visits to a dietician when a diet is prescribed. Patients ought to have the opportunity to change the foods of their diet and have to be informed regularly whether the measures are producing the expected change. For instance, if a patient prefers meat, protein-free bread can be recommended. If a patients does not like protein-free bread, other protein-containing compounds of the diet have to be restricted.

Initially, a low-protein diet will certainly impair the quality of life, as shown by the proportion of dissatisfied patients at 3 months and 6 months. However, the proportion dissatisfied fell every 3 months. Thus, patients appear to get used to a moderately low-protein diet.

Our trial does not elucidate the mechanism by which a low protein diet retards the progression of chronic renal failure. Both urea and phosphate excretion fell in the protein-restricted groups, and serum urea and phosphate fell significantly. The finding of a slight but significant reduction in proteinuria in protein-restricted patients is in accord with previous findings in the remnant kidney model[2] and in man[43], and supports the hypothesis that amino acids and proteins trigger the hepatic secretion of glomerulopressin[12,44], a hormone that reduces the tone of the afferent arteriole[45]. A rise in pressure in the glomerular capillaries will be followed by hyperfiltration and an increase in passage of macromolecules through the glomerular basement membrane; a fall in pressure will have the opposite effect[3,46]. Glomerular hypertension is considered to be a principal factor governing the progression of chronic renal insufficiency[47], and renal diseases accompanied by proteinuria generally have a worse prognosis than those without proteinuria[48].

El-Nahas et al[43] have established that the beneficial effect of low-protein, so-called vasoconstrictive, diets is limited to disorders in which remnant glomeruli are capable of compensatory hyperfiltration. They found that in hypertensive nephrosclerosis, in which ischemia and hypofiltration might predominate, protein restriction did not affect the reciprocal creatinine slope. Similar observations in patients with polycystic kidney disease, contrasting with findings in patients with chronic glomerulonephritis have been reported by Oldrizzi et al[49]. Therefore, further analysis of our data in respect to the underlying diseases is warranted, especially since the faster disease progression found in the younger patients seems to be associated with the greater proportion of cases with glomerulonephritis and reduced renal mass in this group.

The overall reduction in the urea excretion in the two protein-restricted groups was roughly 100 mmol/24h. This implies that a mean reduction of 18 g protein per day had a positive effect in a population with a mean protein intake of 55 (group A2) to 70 (group A1) g per day.

Thus, we have shown that moderate protein restriction introduced early slows down the progression of chronic renal failure or even halts its course. The results might support Brenner's hypothesis on dietary protein intake and the progressive nature of kidney diseases, the hyperfiltration theory[28]. Detailed analysis of our data can show which renal diseases benefit most from dietary protein restriction. Preliminary results indicate that patients with chronic glomerulonephritis do better than patients with nephrosclerosis or polycystic kidney disease. Studies on functional tests to select the patients best suited to protein restriction are warranted.

b) Letter to the Editor

In response to the letters to the editor of Dr. Williams et al and Dr. Westberg et al, whose original text can be found in the appendix to this thesis, the following letter to the editor was published[53].

SIR, We appreciate the comments of several correspondents and others on our article regarding dietary protein restriction in chronic renal failure[52]. Data on the diagnostic groups receiving most benefit will soon be available, preliminary data accord with Dr. Williams and colleagues' findings.

In response to Dr. Westberg, we acknowledge the superiority of the glomerular filtration rate (GFR) as an indicator of progression of renal functional deterioration. To compare our results with previous investigations, however, we used reciprocal serum creatinine values. Regression analysis on the patterns of creatinine clearance were done as well. Creatinine clearance fell significantly in all four groups during the follow-up of 18 months (A1 $p<0.05$; B $p<0.05$; A2 $p<0.001$; C $p<0.01$), but we found striking differences between the slopes in favour of the protein-restricted groups (A1 vs B $p<0.005$; A2 vs. C $p<0.02$). The median losses of creatinine clearance per year were 3.5 (A1), 1.6 (B), 4.2 (A2), and 2.3 (C) ml per min per 1.73 m^2.

Thus the slower increase of serum creatinine in the groups on a protein-restricted diet seems not to be due to a decreased creatinine generation. In our opinion this was already clear from the life-table analyses since significances were reached later than 8-11 weeks[60] for each chosen survival criterion. Moreover, no significant decrease in creatinine excretion was found in group B.

Table III in our paper is apparently being underestimated: it shows that in the control groups 11+3 patients progressed to end-stage renal failure against only 3 in the protein restricted groups ($p<0.01$). The patients who received a transplant all had creatinine clearance above 4 ml per min per 1.73 m^2 (8,9 and 11).

We believe that moderate protein restriction is an acceptable and effective way of delaying functional deterioration in patients with kidney disease. As stated previously, further evaluation is required to detect the patients who will benefit most from dietary manipulation.

Chapter 3

Two years' experience with protein restriction in chronic renal failure

For many years it has been common practice to prescribe protein-restricted diets to patients with advanced renal failure in order to prevent or alleviate uremic symptoms. Arbitrarily, the daily protein intake will be reduced when serum urea concentration exceeds a limit of 20 to 25 mmol/l, regardless of other laboratory data like serum creatinine or phosphate.

Already several decades ago, investigators pointed to a potentially retarding effect of protein-restricted diets in chronic renal insufficiency on the development of end-stage renal failure[51]. But in those years the tremendous prospects of renal replacement therapy became prominent and the ideas of dietary manipulations disappeared.

Renal replacement therapy, however, is very expensive; it consumes an eminent part of our health care budget. Cuts in medical expenses originated a change in attitude in favour of more conservative ways of treatment.

In recent years several, mostly retrospective non-randomised studies confirmed the beneficial effect of dietary protein restriction on the development of an otherwise invariable endstage renal failure[26-34]. The first large prospective randomised study was performed in Groningen, and in this article we present an overview of the now available data of our trial[52-53].

Patients and Methods

All patients visiting the nephrologic outpatient department between January 1, 1982, and April 1, 1984, with a creatinine clearance (CrCl) between 10 and 60 ml/min/1.73 m^2 were included in the study. Excluded were only patients with systemic lupus erythematosus, active vasculitis and Wegener's disease. Patients were stratified according to sex, age (below and above 40 years) and renal function (CrCl below and above 30 ml/min/1.73 m^2). Thereafter, they were randomly allocated to a protein-restricted or a control group.

The quantity of protein prescribed in the restriction groups depended on the degree of renal failure, being 0.6 g/kg/day for patients with a clearance between 31 and 60 ml/min/1.73 m^2 (group B) and 0.4 g/kg/day for patients with a clearance between 10 and 30 ml/min/1.73 m^2 (group C). Their control groups, A1 and A2, respectively, continued their usual diet.

At entry all patients were sent to the dietitian for a detailed diet history. Protein-restricted patients were advised to adhere to the above-mentioned diets. All patients recei-

ved a vitamin/trace element preparation. Patients in group C were supplemented with methionine if necessary, in a dosage of 250 mg t.i.d. orally.

Every three months all patients visited the nephrologic outpatient department. At these occasions many parameters were obtained and stored in a computerized data base. Among them were body weight, blood pressure, hemoglobin concentration, haematocrit, platelet count, serum-pH and serum values of creatinine, urea, calcium, phosphorus, bicarbonate, alkaline phosphatase, total protein, albumin, cholesterol and triglycerides. In 24-hour urine specimens the concentrations of urea, creatinine, protein, sodium, calcium and phosphorus were measured. Patients from groups B and C visited the dietician (the same person during the entire study) every 3 months. Patients from the control groups visited her only on indication. Information retrieved during the interviews, including subjective acceptance of the diet by the patient, were added to the data base.

Patients from the control groups were protein-restricted if their serum urea exceeded values of 25 mmol/l. Dialysis was instituted if creatinine clearance dropped below 4 ml/min/1.73 m^2. Some patients received a cadaveric kidney transplant; never because their creatinine clearance had fallen below 4 ml/min, but because a DR-identical kidney became available.

The 'Groningen Diet'

The diet history was very detailed and included estimations of quantities of energy, protein, sodium, phosphorus, fluid and sometimes potassium intake. Special attention was given to weekends, parties, work, etc., since these are well-known causes of inaccurate calculations.

As protein restriction can have an impact on energy intake, we adapted the diet in case of changes in body weight that were thought to be due to a low energy supply. Thus, the mean energy intake in all groups remained roughly 150 kJ per kg body weight.

The protein content of the diet was composed of proteins of high biological value. In young patients with higher demands of energy, this sometimes gave troubles. Especially if they were from the group of patients with the strongest restriction, e.g. 0.4 g/kg BW, sometimes the advice to eat low-protein bread was given.

Calculation of amino acid patterns[54] in strongly restricted patients often showed methionine deficiency. The above mentioned methionine supplementation was given here.

Most patients were already sodium-restricted, in most cases to six gram per day. To achieve a standardized sodium intake, we introduced the concept of sodium chloride-powders, each sachet containing one gram, so that the patients could decide themselves in what kind of (salt-free) food they wished to add salt. With protein restriction a phosphorus restriction is concomitant.In group C this amounted to 500- 800 mg/day. Every patient was

advised to drink at least 2,000 ml of fluid per day. It appeared from the diet history that especially old people drink less than 1,500 ml per day.

The dietician supplied the patients with a list of the protein content of usual all-day food. A variation list was added as well as a list with products for which no limitation in intake was necessary.

At each follow-up visit, the patients were instructed how to go on with the diet. At the first visit after 3 months, some patients in the protein-restricted groups had lost weight. Therefore, these patients were supplied with 500-800kJ more than calculated from the initial energy demands.

Patients eating low-protein bread sometimes developed a strong aversion against this product. If so, they received a new diet in which normal bread was supplied, but with extra restriction of other proteins, especially from animal sources.

From the urea excretion at the previous visit, the protein intake was calculated. The dietician was informed about the result and used it to motivate the patient to continue the advised diet.

Statistical analysis

From the figures of Maschio et al in their retrospective study[16], we calculated that a first statement about the usefulness of the diet could be made after 18 months. Therefore, our statistical analyses were performed 24 months after the start of the study. The way data on renal insufficiency should be evaluated is still controversial[55]. There is no consensus as to whether a linear regression model or survival analysis establishes the most valid way of showing differences. Thus, we performed statistical tests in various ways.

The ultimate effect of the diet can only be shown after a prolonged period of follow-up after which simply a calculation is made about how many patients entered into dialysis or died due to end-stage renal failure in each subgroup. This, however, requires a follow-up of approximately 5 to 10 years.

Another way of analysing the data is to use survival statistics. Persistent increases in serum creatinine concentrations of for instance 10 or 20% are scored as being a non-survival criterium. Log rank analysis then can disclose statistically significant differences.

A third way of analysing the differences is to use linear regression models. Since in renal failure CrCl tends to follow a linear course towards end-stage renal disease[56], one can use this model to scan for statistically significant differences between patients on a diet or not. The most reliable, but from a practical point of view difficult way of treating data, is to use the patient as his own control. If there are positive effects of the diet, there should be a change in slope after institution of the particular diet.

All above outlined methods were used on our data. We will discuss them separately.

Results

228 patients entered the study before January 1, 1984, and thus were followed up for at least three months. A majority (149 patients) had a follow-up of at least 18 months. The diagnosis distribution is given in Table I. Median age at entry was 47.8 years (15-73 years).

Table I Distribution of renal diseases over the protein-restricted (B and C) and the control groups (A1 and A2).

	A1+A2	B+C	%
1. Unknown	2	5	3.1
2. Membranous glomerulopathy	6	6	5.3
3. Membranoproliferative glomerulonephritis	2	3	2.2
4. Focal glomerulosclerosis	13	17	13.2
5. IgA glomerulopathy	6	3	3.9
6. Glomerulonephritis (other types)	7	12	8.3
7. Adult polycystic kidney disease	4	10	6.1
8. Unilateral agenesis	3	2	2.2
9. Unilateral nephrectomy	6	7	5.7
10. Hypoplasia/dysplasia	1	2	1.3
11. Pyelonephritis	7	6	5.7
12. Alport's syndrome	2	1	1.3
13. Nephrosclerosis	15	11	11.4
14. Analgesic nephropathy	14	10	10.5
15 Interstitial nephritis	3	3	2.6
16. Reflux nephropathy	7	5	5.3
17. Other urologic disorders	6	4	4.4
18. Miscellaneous (amyloidosis, diabetes etc.)	6	11	7.5
	110	118	100

In the control groups three patients died due to end stage renal failure; they had refused dialysis. Moreover, in the control groups 11 patients became dialysis dependent against only three in the protein-restricted groups ($p<0.01$).

Comparison of the survival curves (figure 1) showed statistically significant differences in favour of the protein-restricted patients ($p<0.01$). After 24 months 58% of the protein-restricted patients from group B had a serum creatinine that was still below a 10% increase compared with the initial value against only 22% in group A1. In groups C versus A2 this was 42 against 5%. Statistically significant differences were also found if a 20 or 30% increase in serum creatinine concentration was used as the non-survival criterium.

The linear regression model on median creatinine clearance is shown in figure 2.

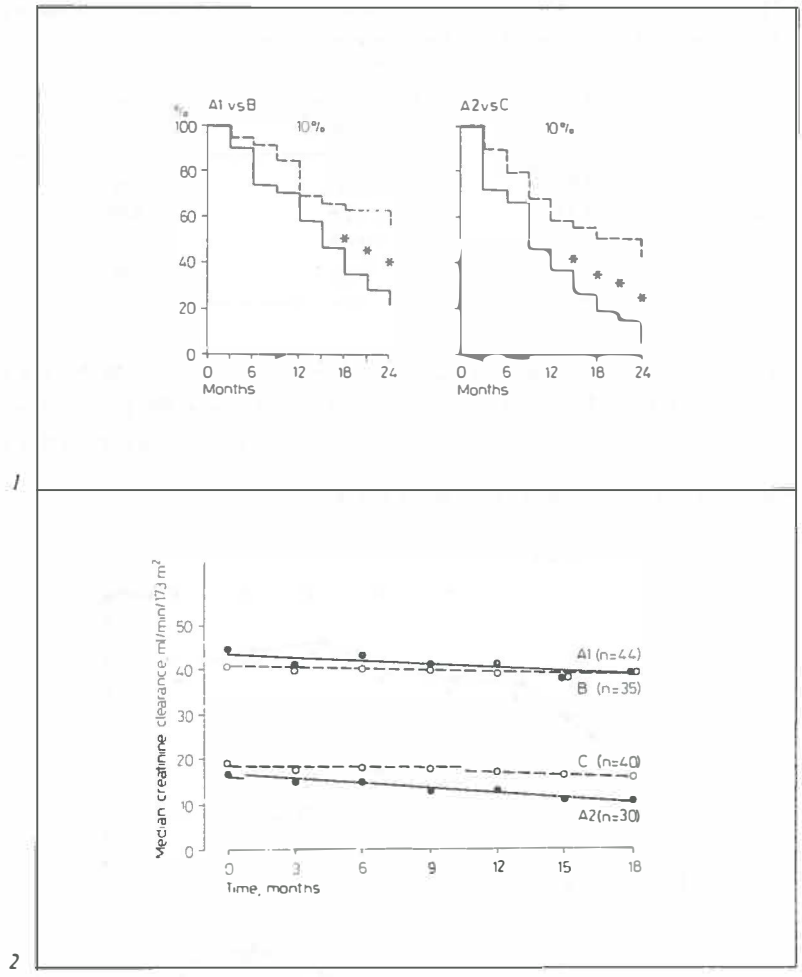

Figure 1 Survival curves for group A1 versus group B and group A2 versus group C with a persistent 10% increase in serum creatinine concentration as the non-survival criterion. Solid lines = control groups; broken lines = protein-restricted groups. *p<0.01.

Figure 2 Relation of creatinine clearance with time (in months) in patients followed for at least 18 months (n = 149). Regression equations: (Y = creatinine clearance: X = time).
A1: Y = 43.6 - 0.29 X; r = –0.85; p<0.05. B: Y = 40.8 – 0.13 X; r = –0.87; p<0.05.
A2: Y = 16.8 - 0.35 X; r = –0.97; p < 0.001. C: Y = 19.2 – 0.19 X; r = –0.93; –p< 0.01.
Slope difference: A2 against C: p< 0.02: A1 against B: p< 0.005.

For the other approach, using patients as their own control, we needed data over a prolonged period before inclusion in the trial. From 151 patients we had creatinine clearance values of at least six months before randomisation. We calculated the slopes of changes in creatinine clearance in these patients before and after stratification. The results are shown in Table II.

Table II Median slopes of decline of creatinine clearance before inclusion and the median changes of the slopes after inclusion in the four patient groups.

	Median slope before	Median change of slope	p value
Group A1 (n=48)	-0.23	-0.03	ns
Group B (n=48)	-0.34	+0.29	<0.05
Group A2 (n=27)	-0.24	+0.09	ns
Group C (n=28)	-0.34	+0.21	<0.01

The slopes of decline in creatinine clearance before entry of the study did not differ between the groups A1 and B, and A2 and C, respectively. Only in the protein-restricted groups B and C there was a significant change in the slope after inclusion in the study.

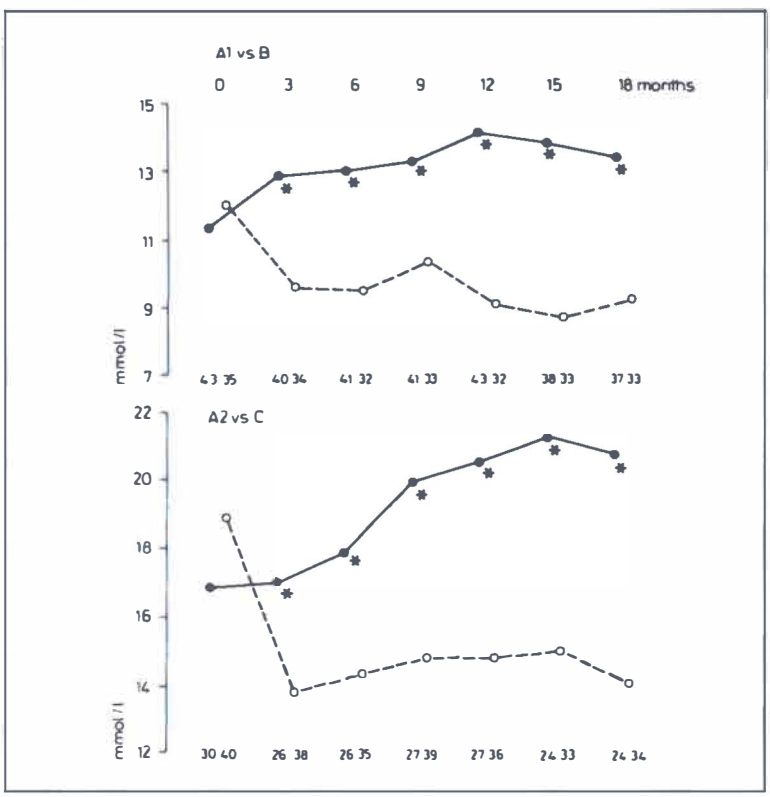

Figure 3 Median serum urea of patients followed for at least 18 months. Solid lines = Control groups; broken lines = protein-restricted groups. *p<0.01. The number of available data in each group at any moment is shown.

Protein-restricted patients and control group patients showed no differences in blood pressure, body weight, serum concentrations of calcium, alkaline phosphatase, uric acid and albumin. The diet appeared to have a considerable reducing effect on serum urea (figure 3) and serum phosphorus (although restricted patients used significantly less phosphate binders).

Proteinuria decreased significantly in the protein-restricted patients whereas the control groups showed no significant changes.

Did patients actually adhere to the advised diet? In figure 4 it is shown that the urea excretion at the start of the study was the same in group A1 and B, and in group A2 and C. The average urea excretion appeared to fall significantly in protein-restricted groups, whereas no changes in urea excretion appeared in the control groups (Figure 4 and Table III). Thus, it could be shown that the patients as a group actually did reduce their protein intake if advised so. From the diet histories it could be learned that patients got used to their diets. At three and six months follow-up the ratio between fair and bad acceptance of the diet was roughly 2/1. At 18 months this ratio was 7/1.

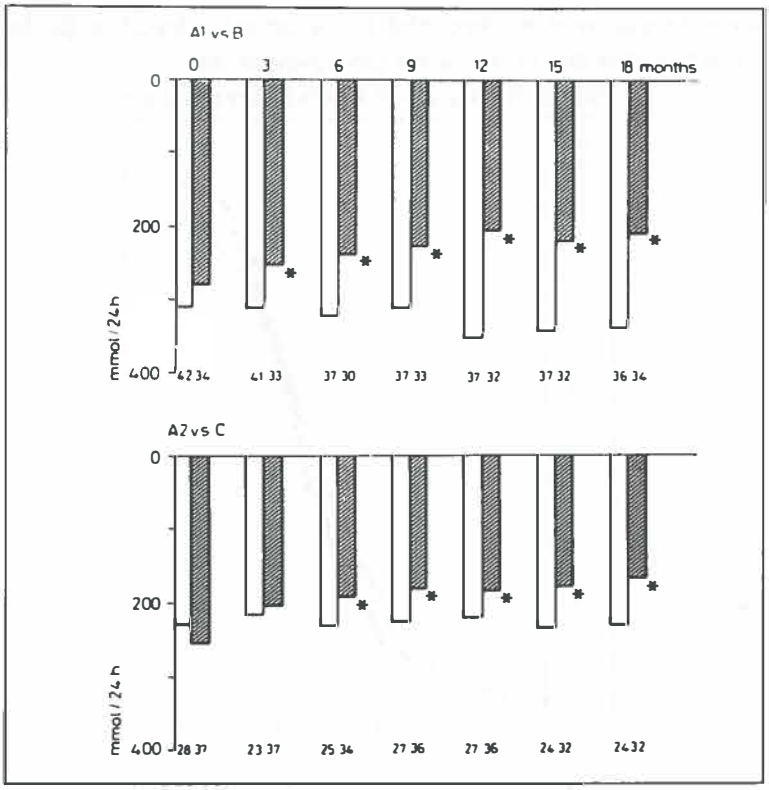

Figure 4 Median 24-hour urinary urea excretion of patients followed for at least 18 months. Open bars = Control groups; hatched bars = protein-restricted groups. *p<0.05. The number of available data in each group at any moment is shown.

Table III Median values of average daily urea excretion before inclusion and the median change in average daily urea excretion after inclusion in the four groups.

	Median average urea excretion before entry mmol/l	Median change in average urea excretion	p value
Group A1 (n=48)	317	+6	ns
Group B (n=48)	305	-86	<0.01
Group A2 (n=27)	231	+8	ns
Group C (n=28)	267	-79	<0.01

It is well-known that protein-restricted diets don't have effect in every patient-category[15,49]. In figure 5 the cumulative proportions of the individual slopes of patients with a follow-up of at least nine months after inclusion are plotted. Three conclusions can be made in regard of this graph:

a) the median slope in the protein-restricted patients is less negative than that in the controls,
b) the observed variation of the slope (both biological and technical) in the diet population is much smaller than in the control group patients, and

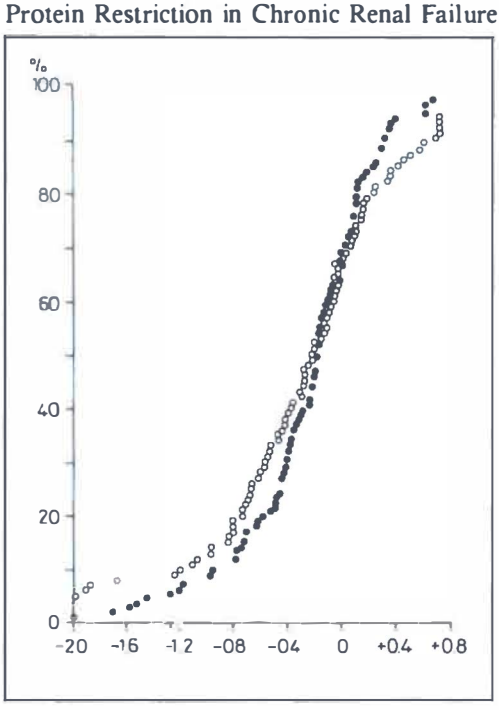

Figure 5 Cumulative proportions of slopes of change in creatinine clearance in protein-restricted patients (closed circles) and control patients (open circles). On the Y-axis the cumulative percentage, on the X-axis the slope of change in creatinine clearance.

c) about one-third of both populations shows a positive slope (=improvement of renal function)

Regarding the subpopulations with a positive slope, one might get the impression that protein restriction prevents improvement of renal function, as the median positive slope in the diet group (0.20) tends to differ from that in the control group (0.37; $p<0.07$). Such a conclusion, however, would be correct only if the variation of slope in an individual was far less than the variation of slope observed in the whole population.

The 10th and 90th percentiles of the slopes in the 75 patients from groups A1 and A2, mentioned in Table II, before inclusion were -2.82 and +1.17, respectively. The same percentiles of the changes in slope after inclusion were -1.28 and +2.43, respectively. This indicates that the observed variation in slope in the population almost exclusively arises from the observed variation in individual slopes.

Looking back to figure 5, the conclusion is that in patients with renal insufficiency intervals of functional improvement are followed by twice as long intervals of decreasing function. Therefore, the suggestion that protein restriction is less beneficial in those patients with a positive slope is incorrect. On the contrary: out of the 151 patients mentioned in Table II, 36 had a positive slope before inclusion, with a median value of 0.18. The 22 of them belonging to the control groups A1 and A2 experienced a huge change in slope after inclusion (-0.65; $p<0.01$), whereas the 14 patients receiving the diet did show only an insignificant change (-0.11; N.S.).

To further elucidate the effect of the diet in the various renal diseases the original diagnosis groups were subdivided into five main clusters (Table IV). The diagnosis polycystic kidney disease is omitted from this table since the number of patients in this subgroup is low if compared to the others.

214 patients with a follow-up of at least six months were used in the computations. For each individual the slope of change in creatinine clearance against time after inclusion was used. Two tailed p-values were obtained by means of a non-paired Wilcoxon test. Median slopes of the control patients versus those of the restricted patients are given. It is evident that patients with glomerulonephritis responded very well to the diet, whereas patients with adult polycystic disease showed even a more rapid deterioration (not shown). However, before stating that protein restriction is harmful to these patients, it should be realized that the number of individuals in this subgroup is low and the period of follow up might be too short for such a conclusion.

Table IV Median slopes of six diagnosis clusters without and with protein restriction.

Cluster		Slope without protein restriction	Slope on protein restriction	p value
1	Glomerulonephritis (2-6)[1]	-0.47 (n=30) (-3.93 to 0.74)[2]	-0.17 (n=39) (-1.44 to 0.91)[2]	<0.01
2	Reduced renal mass (8-10, 16)	-0.30 (n=17) (-1.07 to 2.97)	-0.27 (n=16) (-1.27 to 0.68)	ns
3	Interstitial nephritis (11. 14, 15)	-0.10 (n=22) (-0.73 to 1.00)	-0.13 (n=15) (-1.70 to 0.30)	ns
4	Nephrosclerosis (13)	+0.03 (n=14) (-1.67 to 0.73)	+0.20 (n=11) (-0.77 to 0.63)	ns
5	Rest group (1, 12, 17, 18)	-0.07 (n=16) (-2.00 to 0.35)	-0.07 (n=21) (-4.60 to 0.91)	ns

ns = Not significant (two-tailed Wilcoxon test).
[1]Under the cluster name the diagnosis numbers from Table I to show how the clusters were composed.
[2] Ranges between brackets.

Discussion

In this study (follow-up period maximally 2 years), we have shown that dietary protein restriction to 0.4-0.6 g/kg/day leads to a retardation of the progression of chronic renal failure. The outcome confirms previously published retrospective and prospective, non-randomised trials[26-34].

Linear regression analysis indicated that the median loss in creatinine clearance in the control groups amounted to 3.5-4.2 ml/min/1.73 m^2 per year against 1.6-2.3 ml/min/1.73 m^2 in the protein-restricted groups. The slower increase of serum creatinine in the protein-restricted groups, as expressed in our survival analysis, therefore is not due to a decreased creatinine generation by loss of lean body mass or diminished meat consumption.

The overall reduction in progression was mainly caused by the response to the diet in patients with glomerulonephritis. However, since protein restriction initially appears to decrease glomerular filtration rate[57], apparently by taking away the stimulus that is responsible for the hyperfiltration, one can postulate a beneficial effects even in the other groups after a sufficient period of follow-up. This hypothesis is supported by our finding that protein restriction can prevent the marked fall in function in those patients that were improving their function (through increasing hyperfiltration?) originally.

The average spontaneous rate of progression in various renal diseases is disperse. Patients with chronic glomerulonephritis are known to develop end-stage renal failure much faster than patients with other forms of renal disease. This is also shown by the slo-

pes of the non-restricted patients in Table IV. It can therefore be expected that a response to the diet appears in first instance in the former group. A longer follow-up, probably in the range of 5 years, is warranted for the other forms of renal disease.

Reviewing two years experience with the diet, no harmful effects of the diet were noted. An initial weight loss could be counteracted by supplying more energy. Serum albumin did not change. Patients got used to their diets, most probably a merit of the frequent visits to the dedicated dietician who changed the diet if the patient wished so.

In conclusion, we have shown in this study that protein restriction is a worthwhile method to delay functional deterioration in chronic renal failure, provided that support of an interested dietician is on-hand. Significantly more patients of the non-protein-restricted groups became dialysis-dependent. Log rank analysis on survival with various non-survival criteria showed statistically significant differences in favour of the protein-restricted groups. Regression analysis of the slopes of creatinine clearance against time revealed a significant slower progression in the protein-restricted groups. In those patients of which a preceding slope could be calculated , a significant positive change was found.

Another conclusion is that future studies should have a follow-up of at least four to five years in order to disclose the effect of the diet in slowly progressive diseases like polycystic kidney disease, where taking away the stimulus for hyperfiltration might initially lead to a fall in glomerular filtration rate.

Chapter 4

Protein restriction in chronic renal failure: correlation between creatinine clearance and the reciprocal serum creatinine

Introduction

In the rapidly expanding area of protein-restricted diets to prevent progression of chronic renal failure, there is need for a reliable indicator to assess changes in renal function reflecting the treatment effects. The best way to establish renal function is probably estimation of the glomerular filtration rate (GFR) by using inulin or radio-isotopes.

In clinical practice, calculation of the creatinine clearance (CrCl) as a measure of the GFR is widely used. In the outpatient setting, however, the urine collection can make it an uncertain parameter.

In 1976 Mitch et al. devised a simple method of estimating progression of chronic renal failure by using reciprocal serum creatinine (SCR) values of a patient[56]. As the reciprocal values are thought to be linear with time, the progression of renal failure may be estimated as the slope of the plot of these two variables.

Thus, the effect of a particular therapy can be assessed by calculating the slopes of the reciprocal values before and after start of treatment. In the ensuing years, this approach has been widely used for the evaluation of the influence of low protein diets on the rate of progression in patients with chronic renal failure[58-61]. On the other hand, however, the applicability of this approach seems to be doubtful, since some statistical prerequisites of this method cannot be met by these biological data[30,63,64]. Mathillas et al. demonstrated that serum creatinine measurement may give a falsely positive image of the progression of renal failure compared to radio-isotope methods[65].

As creatinine clearance is supposed to be decreasing linearly with time in patients with chronic renal failure, and as the same is assumed for the reciprocal serum creatinine values with time, there should be a correlation between the slopes calculated from these variables. Very little is known whether this relationship depends on some forms of treatment, i.e. dietary manipulations. Therefore, the aim of the present study is to assess the correlation between the slopes calculated for the creatinine clearance over time and the reciprocal serum creatinine values over time in patients on a free and a low protein diet.

Patients and Methods

The data were taken from a large prospective randomised trial, which has been described in detail elsewhere[52,71]. The 228 patients of this study were divided into four groups:

A1: CrCl 31-60 ml/min;no protein restriction,
B: CrCl 31-60 ml/min; 0.6 g protein/kg/day,
A2: CrCl 10-30 ml/min; no protein restriction, and
C: CrCl 10-30 ml/min; 0.4 g protein/kg/day.

All patients (n=228) were examined every three months; blood and urine samples were collected at these occasions. Only data of patients with a follow-up of 9 months or more (n=207) were selected for the computations. For every individual patient a slope of change in serum creatinine against time was calculated as well as a slope of change in creatinine clearance against time. The reliability of a calculated slope is expressed by the R-value, in fact a correlation coefficient, reflecting the possibility that a given point lies on the calculated regression line. To be more precise, the square value of R: R^2 can be used, and this is the parameter we used in the present study.

Next to calculating correlation coefficients in the entire patient population, two other approaches were employed: one using only those patients whose slope of change in creatinine clearance was characterized by a $R^2 > 0.5$ (R^2 CrCl) and one for those patients whose slope of change in reciprocal serum creatinine against time was >0.5 (R^2 SCR). This restriction excludes data from patients with a poor correlation between the variables clearance and reciprocal values, and time.

For the statistical analysis the SAS program package was used[66].

Results

Plotting slopes of all 207 patients, a diagram as shown in figure 1 was obtained. Figures 2 and 3 show scatter plots for A1 versus B, and A2 versus C.

After plotting slopes against each other, a linear regression analysis was performed and the coefficient of determination (R^2, squared correlation coefficient) was calculated. These results are given in Table I.

In the group of patients with moderately impaired renal function (A1), no correlation between the slopes of reciprocal creatinine and creatinine clearance could be detected. A good correlation existed in the group of patients with poor renal function on an unrestricted diet (A2). In the groups on a low protein diet the correlation was in between these two groups. The wide range between the confidence limits in figures 1-3 demonstrates the wide scattering of data.

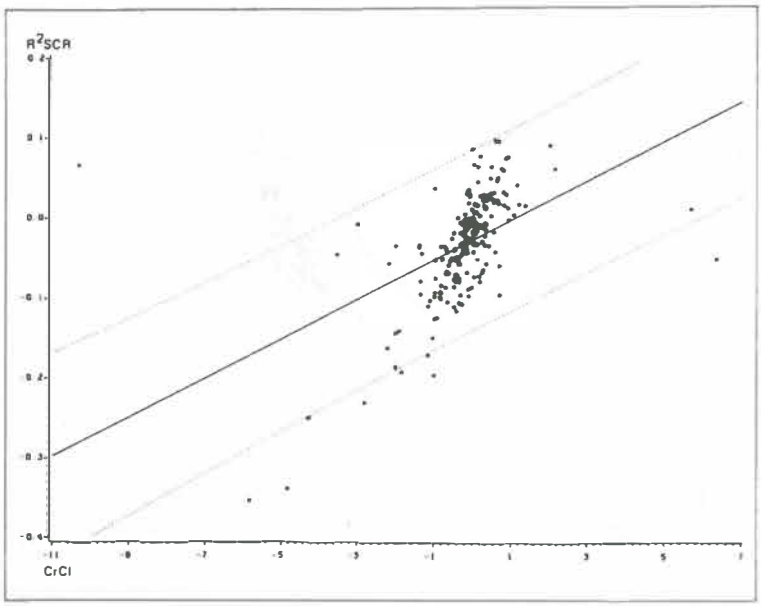

Figure 1 Slopes of creatinine clearance against time versus slopes of reciprocal serum creatinine against time. A regression line is drawn. Dashed lines represent the 95% confidence interval.

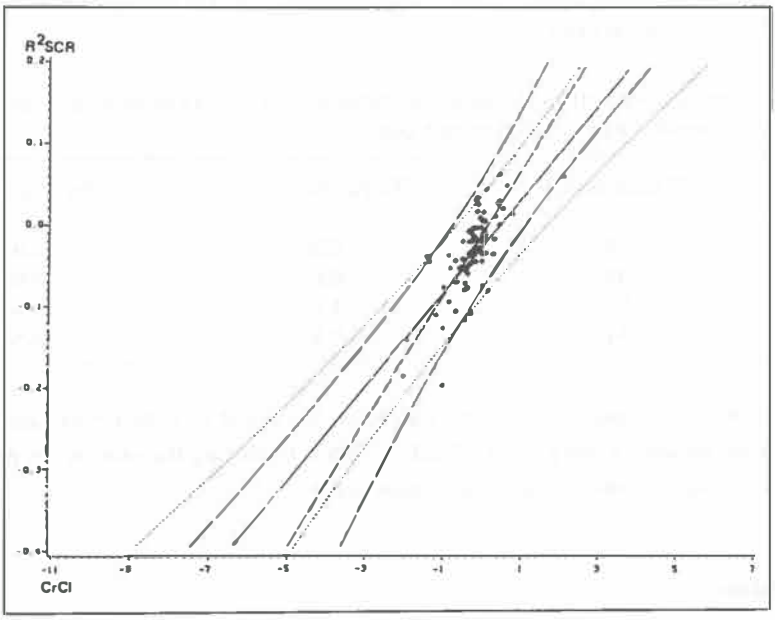

Figure 2 For patients with initial creatinine clearance between 10 and 30 ml/min: slopes of creatinine clearance against time versus slopes of reciprocal serum creatinine against time. Drawn line and stars represent patients without a diet, rough-dashed line and squares represent the diet population. 95% confidence intervals are given.

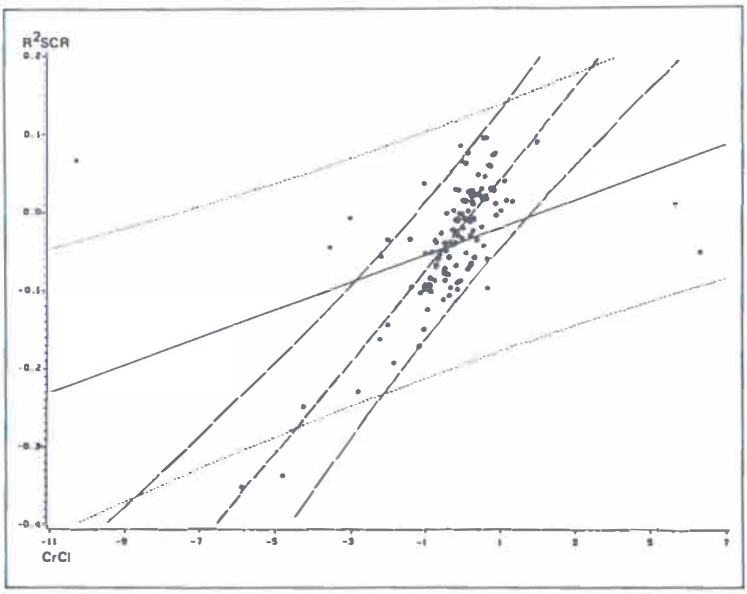

Figure 3 For patients with initial creatinine clearance between 31 and 60 ml/min: slopes of creatinine clearance against time versus slopes of reciprocal serum creatinine against time. Drawn line and stars represent patients without a diet, rough-dashed line and squares represent the diet population. 95% confidence intervals are given.

Table I Correlation coefficients between slopes of creatinine clearance over time and those of the reciprocal serum creatinine over time.

Group	no R^2 limit (n=207)	R^2 CrCl>0.5 (n=78)	R^2SCR>0.5 (n=81)
A1	0.19	0.52	0.81
B	0.48	0.72	0.62
A2	0.65	0.73	0.67
C	0.47	0.40	0.43

Restricting the analyses to slopes with R^2>0.5 resulted in higher correlation coefficients for all groups except group C (Table I). Thus, restricting the analyses of patients of group C to a high R^2 did not affect the relationship.

Discussion

In this study the relationship between slopes of creatinine clearance over time and slopes of reciprocal creatinine values over time has been analysed. The best relationship existed in the group of patients on a free diet having a poor renal function. A very weak correla-

tion was found in patients on a free diet and a moderately impaired renal function, whereas all patients on a low protein diet exhibited a relationship irrespective of renal function. Restricting the analysis to patients with a high R^2 calculated for their individual regression lines, resulted in a higher correlation between the slopes in all groups of patients, except the group on a low protein diet having poor renal function.

As Mitch et al[56] and Rutherford et al[67] have pointed out, reciprocal serum creatinine values of a patient with chronic renal failure linearly decrease over time as creatinine clearance does. Therefore, the slopes calculated for these decreases should correlate. Their method assumes that the degree of correlation is the same at all levels of renal function and under different treatment modalities, e.g. low protein diets. From the data presented in Table I, however, it becomes clear that this relationship is highly dependent on the level of renal function and the mode of therapy.

The poor correlation (R^2=0.19) in group A1 (moderately impaired renal function, unrestricted diet), might be explained by several factors: at low creatinine values the reciprocal data exhibit a wide scattering of data and only a poor linear relationship with time[6,7]. It is well known that the relationship between serum creatinine and GFR is not linear, but has a hyperbolic character. The consequence is, that in patients with glomerular disorders serum creatinine is unlikely to increase overtly unless GFR has fallen by at least 50% below normal values. Using reciprocal transformation, which has a non-linear background, does all but correct this imbalance. At the lower data range, the reciprocal transformation increases the distance between two points, whereas at a higher level the distance is decreased.

Thus, a minor variability of no medical significance, e.g. caused by meat ingestion, results in a statistically important data change. Furthermore, one has to keep in mind that during an observation period of nine months a significant decrease in renal function does not necessarily occur in these patients. Therefore, the calculated slope is somewhat speculative, i.e. it can be changed significantly by a new data point. These facts may explain the vagueness of the calculated regression lines/slopes at this level of renal function.

Correctness of this viewpoint is also supported by the finding of a high R^2 (0.81) in group A1 after the exclusion of all slopes with a corresponding $R^2<0.5$ for the corresponding reciprocal slope. Under these selection criteria only 'valid' slopes are used for the analysis.

The reduction of meat ingestion in group B might result in less variability of serum creatinine, as serum creatinine is in part derived from exogenous sources, e.g. meat[60]. The low level of correlation in group C can be explained by the slower progression of the disease and the resulting less negative slope calculated for this limited observation period.

The highest degree of correlation has been obtained in patients of group A2 who exhibit the most rapid progression rate and the highest serum creatinine values, resulting in less scattering after reciprocal transformation. Thus, only in this group of patients slopes of reciprocal serum creatinine values are representing what they are supposed to do.

Alternatively, creatinine clearance may be employed. Using creatinine clearance, however, has several disadvantages: by using two kinds of laboratory data (serum creatinine and creatinine excretion), an extra systematic error is introduced. Patient compliance is extremely important and in situations where the protein intake is varying, unexpected changes in creatinine clearance can occur. Creatinine clearance is known to overestimate the real GFR because of tubular secretion of creatinine[59]. From a statistical point of view this becomes more important in the patient population with the lowest GFR. Furthermore, in the presence of glomerulopathy, this tubular secretion of creatinine increases significantly as renal failure progresses. Comparative studies have shown that this renders the creatinine clearance less valuable as thought[68].

The superiority of GFR determinations by means of marker infusion must be acknowledged. For large clinical trials, however, the method is unsuitable. Radioactivity limits its use and in outpatient departments it is much too expensive and time-consuming.

This leaves us to the creatinine clearance. In planning large, prospective clinical trials on the use of protein-restricted diets, we have to rely on this parameter. It is cheap, can be performed in every hospital and with good instructions and well-performed dietary counseling it can be reliable.

In summary: the presented data demonstrate that slopes calculated for individual creatinine clearances poorly correlate with slopes calculated for reciprocal serum creatinine data. The relationship is dependent on renal function and dietary treatment. Therefore, use of plots of reciprocal serum creatinine against time should be discouraged for the evaluation of the progression of chronic renal disease, especially in case of applying low protein diets.

Chapter 5

Relationship between proteinuria and response to low protein diets early in chronic renal failure

Summary

In a prospective, randomized trial on the use of protein-restricted diets in chronic renal failure the impact of the protein intake on proteinuria was investigated. Furthermore, the effect on the progression rate of renal disease in patients with low versus high proteinuria was assessed. It is concluded, firstly, that protein restriction reduces protein excretion substantially. Secondly, over 18 months follow-up, especially patients with low protein excretion appeared to respond to the diet. In patients with heavy proteinuria the diet might have beneficial effects in the long term.

Introduction

Protein-restricted diets are highlighted in nephrology today. Several retrospective and prospective studies confirmed their beneficial effect on the progression of chronic renal failure[26-34,52,69-71]. If applied early in renal disease, they can postpone or even prevent the need for dialysis.

A major problem at the moment is to identify the right target group since there is a cumulative body of evidence that not all patient categories respond equally well to the diet[15,49,71]. The nature of the underlying renal disease plays a major role and there are studies that suggest an important relationship between proteinuria and the effectiveness of the diet[15]. The latter study, however, was performed with a very limited number of patients, and without a comparable control group. We assessed the relationship of proteinuria versus diet response in a large group of patients included in a prospective, randomized trial on dietary protein restriction in early renal failure.

Patients and Methods

In the original, prospective, randomized trial[52], 228 patients were entered between January 1, 1982, and April 1, 1984. They had been stratified for sex, age and degree of renal function (creatinine clearance, CrCl), after which they were randomly allocated to a protein-restricted (B or C) or a control group (A1 or A2). Thus, four groups were compa-

red: A1 = CrCl 31-60 ml/min, no protein restriction (n=70); B = CrCl 31-60 ml/min, 0.6 g protein/kg/day (n= 66); A2 = CrCl 10-30 ml/min, no protein restriction (n=40); and group C = CrCl 10-30 ml/min, 0.4 g protein/kg/day (n=52).

All patients received a supplementary vitamin/trace element preparation from the time of randomization. All patients visited the outpatient department every 3 months. On these occasions the following objectives were pursued: body weight; blood pressure; haemoglobin; haematocrit; creatinine; urea; phosphate; calcium; alkaline phosphatase; total protein; albumin; cholesterol; triglycerides; venous pH and bicarbonate. Every 3 months the 24-hour urinary excretion of sodium, urea, creatinine, calcium, phosphate, and protein were measured. Patients in the control groups were switched to a protein-restricted diet if their serum urea exceeded levels of 25 mmol/l.

Progression of renal failure is often established as a calculated slope, using a linear regression model on the change of CrCl over time. In the following statistics only patients with a follow-up of 9 months or longer (n=207) were used. If available, data points from at least 9 months before inclusion in the trial were used to calculate pretreatment slopes (n=140).

Two subgroups were created: proteinuria at study entry below or above 1.0 g/24 h. This cut-off point was chosen because the median proteinuria over the entire patient population amounted to 1.0 g/24 h.

A Wilcoxon matched-pairs signed rank test was used to calculate slope changes within the subgroups and to compare proteinuria at 3-months intervals with the inital value. Differences between the subgroups were tested by means of a Mann-Whitney U test. Patients with glomerulonephritis are of special interest because in this study, they appeared to be the best responders to the diet[71]. Therefore, computations were repeated for this diagnosis group.

Results

Patients with initial protein excretion below 1.0 g/24 h (n=94) did not develop any significant proteinuria during the period of follow-up. One hundred and thirteen patients had a protein excretion of more than 1.0 g/24 h at entry. Median proteinuria values of the free-diet group (n=50) and protein-restricted patients (n=63) during the period of follow-up are given in Table I, and graphically presented in figure 1.

At entry, proteinuria in the free-diet patients did not differ from those on a diet. In controls, proteinuria did not change significantly during follow-up when compared to the time of entry. However, in protein-restricted patients, a persistent reduction in proteinuria, already present after 3 months appeared to exist. Intergroup comparisons showed that proteinuria was significantly ($p<0.05$) lower in the protein-restricted patients at 3, 6, and 9 months.

Table I Median proteinuria in patients starting with a protein excretion of > 1.0 g/24h.

Diet	Time, months						
	0	3	6	9	12	15	18
Free	3.2	2.7	2.7	2.6	2.7	2.5	2.9
n	50	48	47	43	41	29	27
Protein-restricted	3.1	1.4 **	1.8 **	2.3 **	2.4 *	2.5 *	1.8 *
n	63	63	59	60	54	46	36

Significant compared to moment 0:* $p < 0.05$; ** $p < 0.005$.

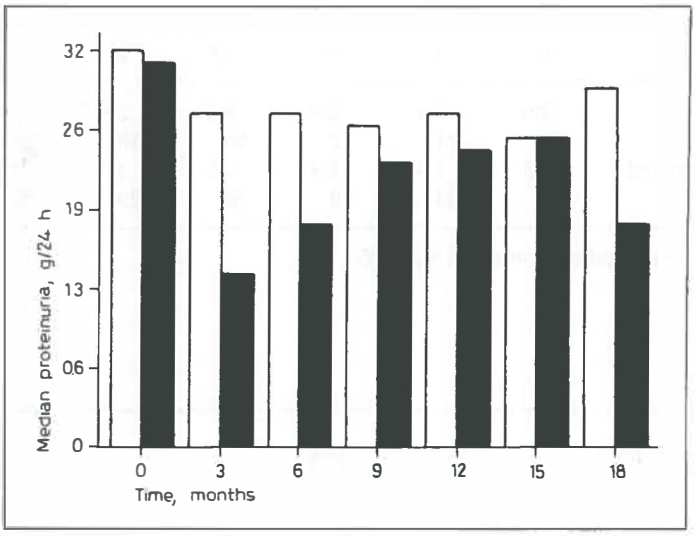

Figure 1 Median urinary protein excretion in patients with an initial proteinuria of > 1 g/24 h. □ = Free diet; ■ = protein-restricted diet.

In Table II the results of slope comparisons are given. It is evident that protein-restricted patients with a proteinuria below 1.0 g/24 h responded with a significant improvement of slope ($p<0.005$), whereas patients on a diet in the group above 1.0 g did show a trend towards improvement ($p=0.07$). Patients on a free diet showed no change of slope.

Restricting the computations to patients with glomerulonephritis, we found 54 patients with an initial proteinuria of more than 1 g/24h. The values during follow-up are provided in Table III, and graphically depicted in figure 2. Again protein-restriction led to a decrease in proteinuria. Control group patients showed no significant changes. Intergroup comparisons showed significant differences ($p<0.05$) at 3, 6, 9, 12 and 18 months. Slope comparisons regarding creatinine clearance are presented in Table IV.

Table II Median slopes of change in CrCl before and after inclusion in the trial: p values for change of slope are given.

Diet		Before	After	p
Proteinuria<1.0 g/24h	Free(n=26)	-0.18	-0.08	NS
	Protein-restricted (n=30)	-0.19	0.01	<0.005
Proteinuria>1.0 g/24 h	Free (n=42)	-0.39	-0.52	NS
	Protein-restricted (n=42)	-0.43	-0.24	NS

Table III Median proteinuria in patients with glomerulonephritis starting with a protein excretion of > 1.0 g/24 h.

Diet	Time, months						
	0	3	6	9	12	15	18
Free	3.0	3.2	3.4	3.9	2.8	2.5	3.0
n	23	21	22	20	18	13	11
Protein-restricted	2.3	1.5 *	1.9 *	2.5	2.0 *	2.1	1.4 *
n	31	33	32	29	29	25	18

Significant compared to moment 0: * $p<0.05$.

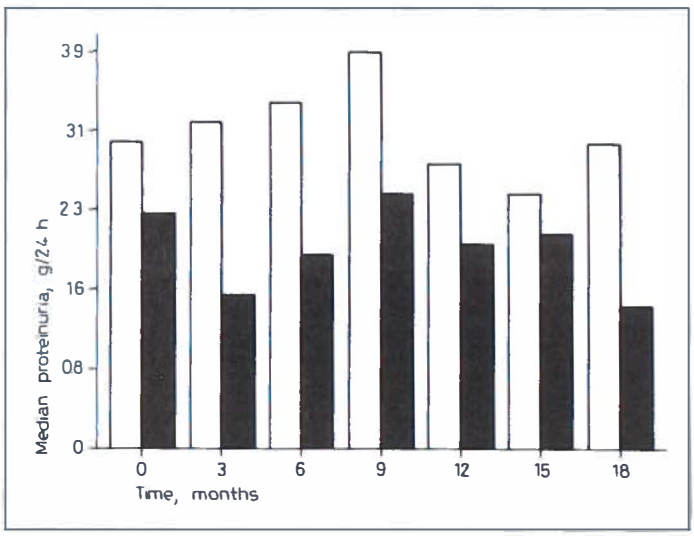

Figure 2 Median urinary protein excretion in patients with chronic glomerulonephritis and an initial proteinuria of >1 g/24 h. □ = Free diet; ■ = protein-restricted diet.

Table IV Median change of slope in CrCl in patients with glomerulonephritis before and after inlcusion in the trial (p values for change of slope are given).

Diet		Before	After	p
Proteinuria<1.0 g/24 h	Free (n=4)	-0.18	-0.26	NS
	Protein-restricted (n=7)	-0.26	0.10	<0.01
Proteinuria>1.0 g/24 h	Free (n=19)	-0.72	-0.60	NS
	Protein-restricted (n=24)	-0.60	-0.36	NS

From 54 patients with glomerulonephritis we had slopes before and after inclusion in the study. Only 11 patients had a proteinuria below 1 g/24 h at entry. Significant changes were reached only in protein-restricted patients with a proteinuria below 1.0 g/24 h. In case of protein excretion above 1 g/24 h, the diet group showed a trend (p=0.07) towards improvement of renal function. Control group patients showed no change in slope.

Discussion

Glomerular hyperfiltration is thought to be one of the main determinants in the progression of chronic renal failure[50]. In rats it has been demonstrated that in case of a reduction in renal mass, the remaining glomeruli compensate for the lost nephrons by means of hyperfiltration[72]. This happens not only in case of (subtotal) nephrectomy[1,73], but also in renal disease, e.g. chronic pyelonephritis[74,75]. Such compensatory hyperfiltration, however, results in a progressively downhill course of renal function, which is characterized by the development of proteinuria, hypertension and diffuse glomerulosclerosis[1,73,74]. Proteinuria might be caused by hyperfiltration-induced, altered size- and charge- selectivity properties of the glomerular wall[42]. The progression towards end-stage renal failure can be slowed down by the institution of a protein-restricted diet which reduces glomerular hyperfiltration[73,74].

The concept of glomerular hyperfiltration implies that remnant glomeruli possess a reserve filtration capacity. In healthy subjects such reserve capacity has been demonstrated[76,77]. In patients with moderate to severe renal insufficiency on the other hand, hardly any or no reserve is left, indicating the existence of glomerular hyperfiltration[76-78]. Protein-restricted diets do not only reduce the glomerular filtration rate[79] and proteinuria[15,80,81], but also have a beneficial effect on the course of renal disease[26-34,52], probably by reducing glomerular hyperfiltration.

As was described earlier[52], we have found that protein restriction retards the progression of renal failure. In the present study, we found a significant reduction in proteinuria in patients on a protein-restricted diet. Noteworthy is, that patients with an initial proteinuria below 1.0 g/24 h did not develop any significant protein excretion during follow-up, irrespective of which group they belonged to. Similar observations were made in patients with glomerulonephritis.

An important finding is, that patients with initial proteinuria below 1.0 g/24 h show the best response to the diet, as expressed by improvement of their CrCl slope. One might conclude that patients with initial heavy proteinuria may have passed their moment of reversible hyperfiltration. However, we showed that a protein-restricted diet causes a significant reduction in proteinuria in this group. Therefore, the explanation for the observation that there only existed a trend towards improvement might be that the period of follow-up was too short.

In summary, the following conclusions can be drawn. First, protein restriction leads to a decrease in proteinuria. Second, at least in the short term, patients with a low proteinuria show a better response to the diet with respect to the progression of renal disease. Finally, to elucidate whether patients with heavy proteinuria may also benefit from the diet, longer periods of prospective follow-up are warranted.

Chapter 6

Protein-restricted diets in chronic renal failure: a four year follow-up shows limited indications

Summary

Several retrospective and prospective studies confirmed the beneficial effect of dietary protein restriction (DPR) on the downhill course of renal function in chronic kidney disease. The long-term results of this therapeutic modality may be different than the short-term effects. In our nephrology outpatient department, a prospective randomized trial has been in progress since April, 1982. In 1984, we reported a general beneficial effect of our diet after two years of follow-up. Two hundred and forty-eight patients with initial clearances between 10 and 60 ml/min entered the trial. Patients were stratified for sex, age and degree of renal insufficiency. One hundred and twenty-nine patients were randomly assigned to a DPR group (0.4 to 0.6 g/kg/day); 118 to a control group. Patients on DPR visited the dietician every three months during the first 24 months of the study; thereafter, as with the controls, the dietician visits were only for specific needs. Urea excretion decreased significantly in DPR patients as a sign of good compliance and stayed at that level, even without frequent visits to the dietician. Biochemical parameters showed no sign of malnutrition. The diet appeared to have a selective effect on the progression rate of renal failure: only patients with primary glomerular disease responded to the diet. Furthermore, there were striking intersex differences. Males showed a more rapid decline towards end-stage renal failure, but responded in a positive way to the diet, whereas female patients did not benefit from the dietary manipulation at all. In patients with adult polycystic kidney disease, the rate of progression of renal failure was entirely related to blood pressure control, whereas in the other diagnostic groups, the regulation of blood pressure played a minor role. In conclusion, after four years of follow-up, we are only moderately optimistic about DPR as a general measure for the management of the progression of chronic renal insufficiency. Such diets should be used in the selected subgroups indicated above. The results from trials of dietary protein restriction can only be validated after a follow-up period of at least four years.

Introduction

Recently, we showed in a prospective randomized trial that moderate dietary protein restriction slows the progression rate of chronic renal failure[52,53,71]. Our finding was in

agreement with numerous, mostly retrospective trials[26-34]. Some studies have suggested that the beneficial effect of a low protein diet (LPD) depends on the underlying nephropathy. El Nahas et al[15] for instance, described an improvement in renal function in patients with chronic tubulointerstitial nephritis, no effect in ischemic nephrosclerosis, and a marginally beneficial effect in chronic glomerulonephritis. Oldrizzi et al[49], in a retrospective study, found the best response in patients with primary chronic pyelonephritis, whereas patients with chronic glomerulonephritis did better than patients with adult polycystic kidney disease. In our patients, we have shown that after a follow-up period of two years, the good response to the diet was almost exclusively due to the beneficial effect in patients with primary glomerular nephropathy[71].

One major deficiency of all studies is the limited period of follow-up. Since initially a LPD appears to decrease the glomerular filtration rate[57], a period of as long as two years may be insufficient to show an effect on the rate of progression. This might be particularly true for patients with diseases that show a slow course to end-stage renal disease, such as adult polycystic kidney disease.

In the present study we provide our data after 48 months of follow-up with special emphasis on the response of the various diagnostic groups and with several statistical approaches including changes in slopes of creatinine clearance against time before and after inclusion in the trial. Other factors that may have impact on the progression rate, such as blood pressure, proteinuria and sex were also studied and compared with the influence of the diet itself.

Many physicians avoid protein-restricted diets because of fear of malnutrition. Several parameters of nutritional status are commonly assessed. Among them are anthropometric data, plasma proteins with short biological half-lives (such as transferrin, cholinesterase, C_3 complement), serum albumin, and plasma amino acid/keto analogue levels. We performed amino acid analyses, including the estimation of their keto-analogues in a subset of our patients.

Methods

All patients visiting the nephrology outpatient department between January 1, 1982, and April 1, 1984, with a creatinine clearance (Ccr) between 10 and 60 ml/min/1.73 m^2, were considered for the trial. Excluded were patients with lupus erythematosus, active vasculitis and Wegener's disease. After obtaining consent, patients were stratified according to sex, age (below and above 40 years) and renal function (Ccr below and above 30 ml/min/1.73 m^2). Thereafter, they were randomly allocated to a protein-restricted or a control group.

The quantity of protein prescribed in the restriction groups depended on the degree of renal failure: 0.6 g/kg/day for Ccr of 31 to 60 ml/min (group B, N=74) and 0.4 g/kg/day for a Ccr of 10 to 30 ml/min (group C, N=56). The control groups were as follows: A1, N=76 (Ccr 31 to 60 ml/min) and A2, N=41 (Ccr 10 to 30 ml/min). Patients in the control

groups continued to eat their usual diet. Thus, there were four main groups in the study. At entry, the dietician obtained a detailed dietary history on each patient. Protein-restricted patients were advised to adhere to the above mentioned diets. All patients received a vitamin and trace element preparation. Those from group C were supplemented with methionine, if necessary, in a dosage of 250 mg t.i.d. orally.

After the initial visit, patients were evaluated by the nephrologist every three months. For patients in the LPD groups, this was combined with a visit to the dietician (the same dietician during the entire study). Patients in the control group visited the dietician only for a specific indication. Starting in January, 1985, all patients were given the same dietary follow-up, which meant visits to the dietician only for a specific indication.

At all visits, the following variables were obtained and recorded in a computerized data base: body weight, sitting blood pressure, leucocyte and erythrocyte counts, hemoglobin, hematocrit, platelet count, serum concentrations of creatinine, urea, uric acid, calcium, phosphate, alkaline phoshatase, total protein, albumin, cholesterol, triglycerides, bicarbonate and venous pH. Twenty-four hour urinary excretion of urea, creatinine, protein, sodium, calcium and phosphate also were measured. Blood pressure, serum calcium and serum phosphate were kept within narrow limits, if necessary, with use of pharmaceuticals.

Patients from the control groups were protein-restricted if their serum urea exceeded 25 mmol/l. Dialysis was instituted if the creatinine clearance decreased below 4 ml/min. Some patients received a cadaveric kidney transplant, not because their renal function prompted to starting hemodialysis, but because a DR-identical kidney became available. In 109 patients who had been followed for a median of 42 months, a blood sample was drawn for amino acid analysis.

Statistical analysis

The optimal approach to evaluate the rate of progression of renal insufficiency is still controversial[82]. Ultimately, the most important parameter is how many patients progress to end-stage renal disease and become dialysis dependent or die. This requires a long period of follow-up. One way of analyzing the data is to use survival statistics. Persistent loss of creatinine clearance of, for instance, 50% is scored as being the non-survival criterion if the initial clearance values in the various groups do not differ. Log rank analysis then can be used to disclose statistical differences[36]. A major advantage of this method is that drop-outs have no influence on the statistics. A problem arises, however, in choosing the right criterion level, since the choice of 50% is strictly arbitrary.

Another way of analyzing the data is to use linear regression models. Since in chronic renal failure, Ccr usually decreases linearly over time, this model might be suitable to test for intergroup differences, especially with regard to changes in median creatinine clearances. However, in long-term trials this method introduces a pitfall, since drop-outs have a tremendous influence on the slope of the linear regression line of the median creatinine

clearances. Patients that drop out because they became dialysis dependent influence the slope of the entire group in a positive way. Hence, this method is only suitable in patient groups with low numbers of drop-outs.

A reliable, but from a practical point of view often difficult way of analyzing data, is to use the patient as his own control. If there are positive effects of the LPD, there should be a persistent change in the slope of the Ccr-decline after institution of the diet.

All of these methods were used for our data analysis. For comparsion of the measured data between the groups, the Mann-Whitney U-test was used. Changes in variables within the groups as well as a change of slope before and after inclusion in the trial were determined with the Wilcoxon matched-pairs signed-ranks test. Differences in frequency distributions were tested by chi-square analysis, corrected for continuity.

Results

Two hundred and forty-eight patients entered the study before April 1, 1984. The distribution after randomization is given in figure 1. One patient had to be excluded after two months because of a concomitant disease, making further study impossible. The various diagnoses are given in Table 1. Median age at entry was 48 years (range 15 to 73 years).

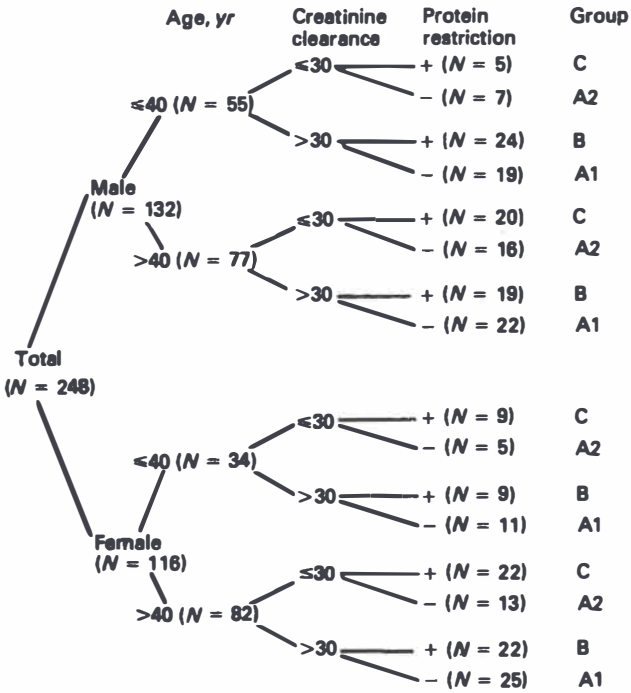

Figure 1 Distribution of patients after stratification for sex, age and renal function (ml/min/1.73 m^2).

Table I Distribution of various renal diseases over the protein-restricted (B and C) and the control groups (A1 and A2).

		A1 + A2	B + C
1.	Unknown	2	7
2.	Membranous glomerulopathy	6	8
3.	Membranoproliferative glomerulonephritis	2	4
4.	Focal glomerulosclerosis	13	18
5.	IgA glomerulopathy	7	4
6.	Glomerulonephritis (other types)	7	14
7.	Adult polycystic kidney disease	4	11
8.	Unilateral agenesis	3	2
9.	Unilateral nephrectomy	6	7
10.	Hypoplasia/dysplasia	1	2
11.	Pyelonephritis	7	6
12.	Alport's syndrome	2	1
13.	Nephrosclerosis	17	11
14.	Analgesic nephropathy	15	10
15	Interstitial nephritis	3	3
16.	Reflux nephropathy	8	5
17.	Other urologic disorders	7	4
18.	Other (amyloidosis, diabetes etc.)	7	13
	Total	117	130

One hundred and fifty-three patients had a follow-up of 36 months or longer. Patients in the four groups were not statistically different with regard to body weight, blood pressure, serum concentrations of calcium, phosphate, alkaline phosphatase, total protein, albumin, uric acid, cholesterol, triglycerides and initial level of proteinuria. (Table 2). Patients from the protein-restricted groups showed significantly lower serum phosphate levels, although they used less phosphate binders ($p<0.05$). Furthermore, the diet substantially reduced proteinuria after 12 and 24 months of follow-up. However, at 36 months of follow-up there were no differences in proteinuria.

Creatinine clearance at entry was not statistically different in group A1 versus B and group A2 versus C. In the control groups, 25 patients progressed to ESRD against only 14 in the LPD groups, thus rendering a significant difference (A1+A2 vs. B+C: $p<0.05$). This was due to the good response in the C-group (Table 3; A1 vs. B: NS, A2 vs. C: $p<0.005$).

Comparison of the survival curves (fig. 2) showed significant differences in favour of those LPD patients who had low initial creatinine clearances (group C; $p<0.025$). For patients with higher initial values, no effect of the diet was established when using a 50% decline in Ccr as the survival criterion. Using a 20% criterion, statistically significant differences in both groups were obtained (results not shown).

Table II Median values of variables measured at entry and after 12, 24, and 36 months for patients with a follow-up of at least 36 months.

(N = 153)

	Diet	0	12	24	36
Body weight *kg*	Pr+	74	72	74	73
	Pr-	70	70	73	72
Blood pressure *mm Hg*	Pr+	140/90	134/90	140/90	140/90
	Pr-	140/90	140/90	140/90	150/90
Hematocrit *%*	Pr+	41	41	40	41
	Pr-	40	42	42	40
Serum calcium *mmol/liter*	Pr+	2.44	2.44	2.43	2.40
	Pr-	2.42	2.46	2.43	2.38
Serum phosphate *mmol/liter*	Pr+	1.08	1.00[b]	1.00[b]	1.08[a]
	Pr-	1.14	1.14	1.16	1.15
Serum alk. phosph. *U/liter*	Pr+	92	84	86	78
	Pr-	81	89	81	80
Total protein *g/liter*	Pr+	68	68	68	69
	Pr-	67	69	67	68
Serum albumin *g/liter*	Pr+	42	43	44	43
	Pr-	42	43	42	43
Serum cholesterol *mmol/liter*	Pr+	6.8	6.6	6.6	6.9
	Pr-	6.6	6.6	6.5	6.5
Serum triglycerides *mmol/liter*	Pr+	2.3	2.6	2.5	2.4
	Pr-	2.2	2.0	2.3	2.4
Proteinuria *g/24 hr*	Pr+	1.5	0.5[a]	1.2[b]	1.5
	Pr-	2.3	2.3	2.9	1.7

Abbreviations are: Pr+, protein restricted; Pr-, free diet.
[a] $P<0.05$. [b] $P<0.005$. Mann Whitney test for group differences.

Table III Patients removed from study (N = 74).

	A2	C	A1	B
Died (due to ESRD)	3	4	7	4
	(2)	(–)	(3)	(–)
Dialysis	17	8	3	6
Transplantation	4	7	–	1
Lost (9 moved, 1 withdrawal)	3	1	3	3
Total	27	20	13	14

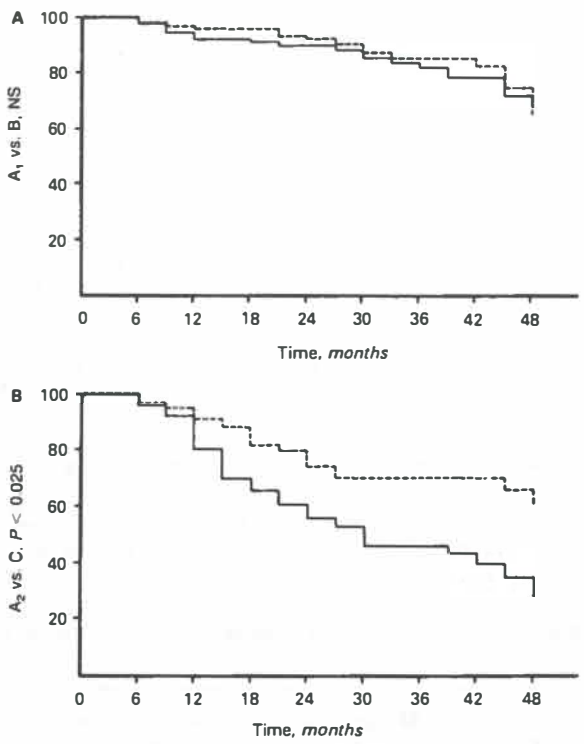

Figure 2 Survival curves for group A1 versus group B (**A**, P = NS) and group A2 versus group C (**B**, P< 0.025) with a persistent 50% loss in creatinine clearance as non-survival criterion. Broken lines = protein-restricted groups, solid lines = control groups.

The linear regression model is shown in figure 3. In this statistical modality, no effect of the diet on median Ccr was noticed in either group.

In 151 of the patients, we were able to obtain creatinine clearance values over at least six months before stratification. We calculated the respective slopes of median creatinine clearance values before and after three months treatment in the trial. The results are depicted in Table 4.

From this table, it again can be concluded that the C group benefited the most. Although in the B group patients a trend towards improvement was noticed, it was not statistically significant due to the wide variability in slopes.

Did patients actually adhere to the prescribed diet? Urea excretion fell significantly during the follow-up period (fig. 4).

Interestingly, even after stopping intensive dietary follow-up, the patients maintained their low urea excretion. This implies that most of the patients became accustomed to the diet, which seemed to make vigorous dietary follow-up less necessary.

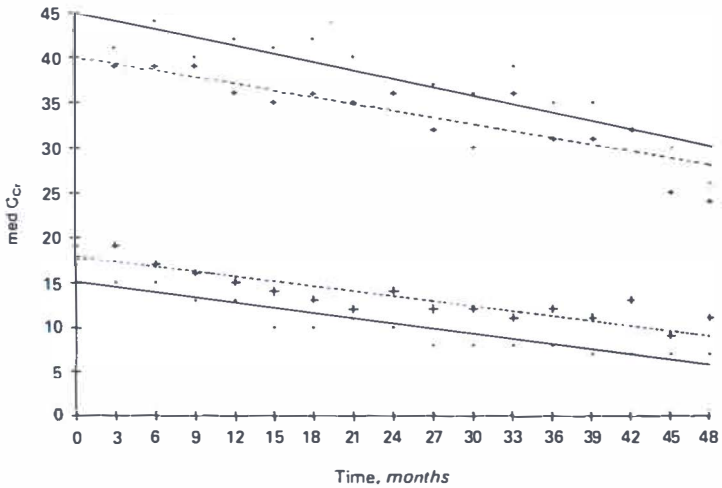

Figure 3 Relation of median creatinine clearance (Ccr) with time in months for the entire patient population (N = 247).
A1, top solid line: Ccr = 45.1 - 0.31 x time: r = -0.90; P<0.05. A2, bottom solid line: Ccr = 15.1–0.20 x time: r = –0.94: P< 0.05. B. top dashed line: Ccr = 40.7 – 0.28 x time: r = –0.88; P< 0.05. C, bottom dashed line: Ccr = 17.2 – 0.16 x time; r = –0.89; P<0.05.

Table IV Median slopes of decline in creatinine clearance before and after inclusion in the four patient groups.

	N	Median slope before	Median slope after	*P* value
Group A1	48	-0.25	-0.20	NS
Group B	48	-0.34	-0.17	0.06
Group A2	26	-0.27	-0.19	NS
Group C	29	-0.29	-0.13	<0.01

To analyze the influence of sex, we compared the slopes of progression over 48 months between male and female patients. The results are depicted in Table 5.

Male patients showed a more rapid decline in renal function as compared to female patients; however, they responded well to the diet, whereas female patients showed no response at all.

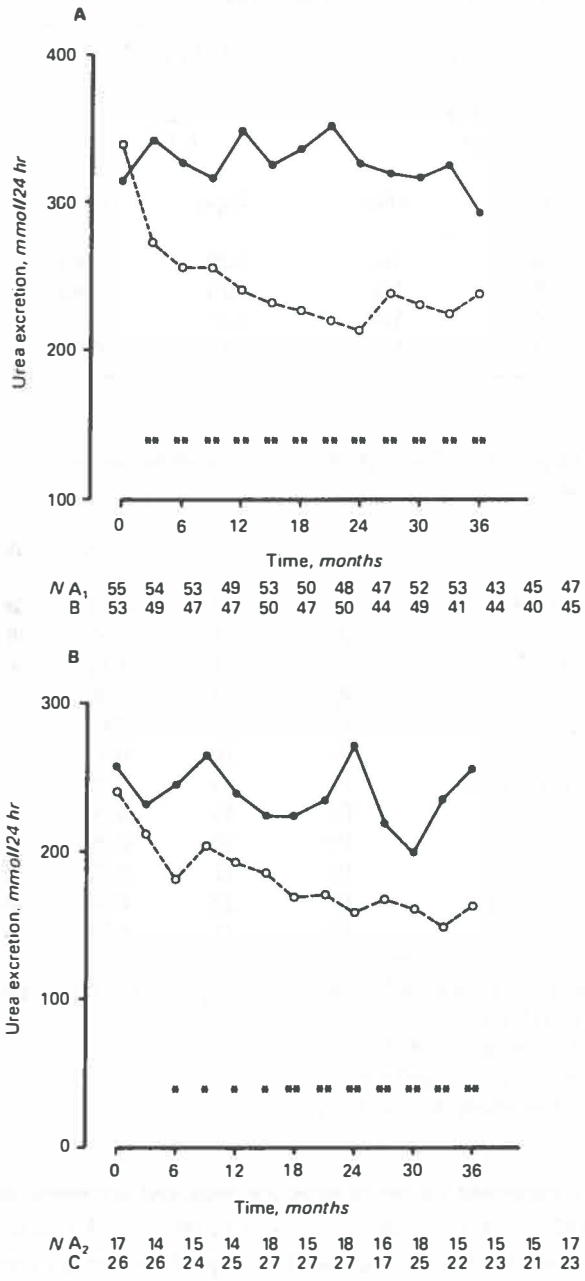

Figure 4 Median 24-hour urea excretion of patients followed for at least 36 months.
A. Symbols are: (●—●) A1; (○—○) B; **P< 0.01 (A1 versus B).
B. Symbols are: (●—●) A2; (○—○) C; *P<0.05; **P<0.01. (A2 versus C).

Table V The influence of sex on the progression rate.

Sex	*N*	Median slope	*P* value
Male	128	-0.22	<0.01
Female	111	-0.17	

Sex	*N*	Diet	Slope	r value	*P* value
Male	66	Yes	-0.20	0.86	<0.05
Male	62	No	-0.24	0.92	
Female	60	Yes	-0.17	0.75	NS
Female	51	No	-0.17	0.74	

Table VI Median slopes of decline of creatinine clearance before and after inclusion in the six diagnostic groups.

	Diet	*N*	Before	After	
Glomerulonephritis (2-6)[a]	Pr+[b]	32	-0.44	-0.16	<0.05
	Pr-[c]	24	-0.27	-0.30	NS[d]
Polycystic disease (7)	Pr+	4	-1.11	-0.37	NS
	Pr-	3	-0.36	-0.07	NS
Reduced renal mass (8-10, 16)	Pr+	12	-0.41	-0.22	NS
	Pr-	10	-0.24	-0.12	NS
Interstitial nephritis (11, 14, 15)	Pr+	9	-0.03	-0.15	NS
	Pr-	15	-0.10	-0.08	NS
Nephrosclerosis (13)	Pr+	10	-0.05	-0.11	NS
	Pr-	11	-0.47	-0.25	NS
Miscellaneous (1, 12, 17, 18)	Pr+	10	-0.33	-0.06	<0.05
	Pr-	11	-0.21	-0.16	NS

[a] Numbers in parentheses under each diagnostic group indicate the diagnoses within that group. as descibed in Table I.
[b] Pr+ are patients on a low protein diet.
[c] Pr- are patients from the control groups.
[d] NS, not significant (two-tailed Wilcoxon test).

In Table 6 data are presented on the progression analyzed according to the diagnostic groups. Patients with glomerulonephritis as well as those in the miscellaneous group benefited from the diet. In the other diagnostic groups, however, no statistically significant improvement in renal function was observed.

The good response of patients with glomerulonephritis prompted us to perform separate statistics for this subgroup. Whereas no significant improvement of the slope of the median Ccr was observed in the total patient group using linear regression analysis, we found such a difference in the patients with glomerulonephritis (fig. 5). In group A2, only

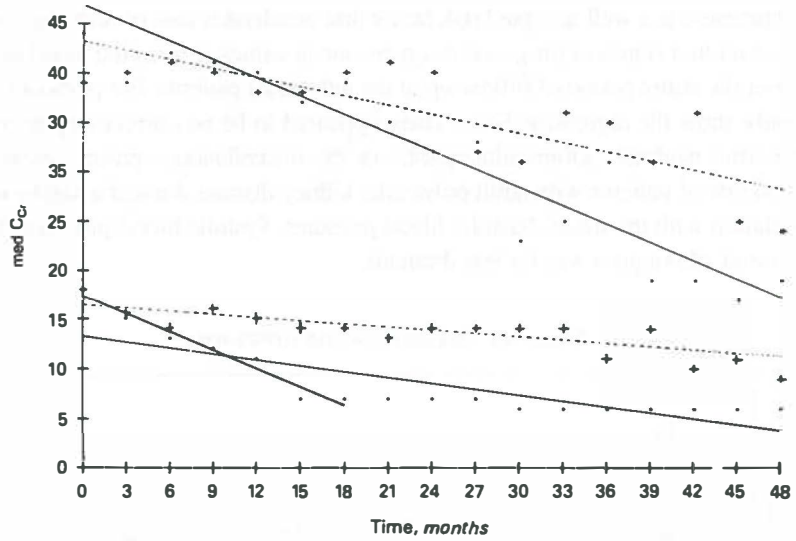

Figure 5 Relation of median creatinine clearance (Ccr) with time in months for patients with glomerulonephritis (N = 83). A1, top solid line: Ccr = 4.57 - 0.59 x time: r = -0.94: P<0.01. A2, bottom solid line: Ccr = 17.3 - 0.61 x time: r = - 0.98: P<0.001. B, top dashed line: Ccr = 43.3 - 0.31 x time: r = - 0.81: NS. C, bottom dashed line: Ccr = 16.0 - 0.11 x time: r = -0.80: NS.

the values up to 18 months were used because of the loss of many patients due to the need to start dialysis.

The influence of the initial urinary protein excretion on the progression of renal disease is controversial. To study this phenomenon, we divided the patients into two groups based on whether urinary excretion was below or above 1 gram of protein per day at the time of randomization. The results are presented in Table 7. Protein restriction had a positive effect in both patient groups. The initial urinary protein excretion thus does not indicate the probability of success with dietary treatment.

Table VII Relationship between initial proteinuria and the progression rate.

			Median slope		
Proteinuria	Diet	*N*	Before	After	*P* value
<1 g/24 hr	Pr+	35	-0.20	-0.11	<0.01
P			NS	<0.01	
	Pr-	29	-0.10	-0.20	<0.05
≥ 1 g/24	Pr+	22	-0.20	-0.12	<0.01
P			NS	<0.01	
	Pr-	25	-0.20	-0.31	<0.05

Blood pressure is a well accepted risk factor that accelerates loss of renal function. In figure 6, we plotted slopes of progression against mean values of diastolic blood pressure derived over the entire period of follow-up in the individual patients. For purposes of clarity, we only show the regression lines. There appeared to be no correlation for patients with interstitial nephritis, glomerulonephritis or the miscellaneous group, whereas the progression rate of patients with adult polycystic kidney disease showed a highly significant correlation with the mean diastolic blood pressure. Systolic blood pressure showed the same trend, although it was far less dramatic.

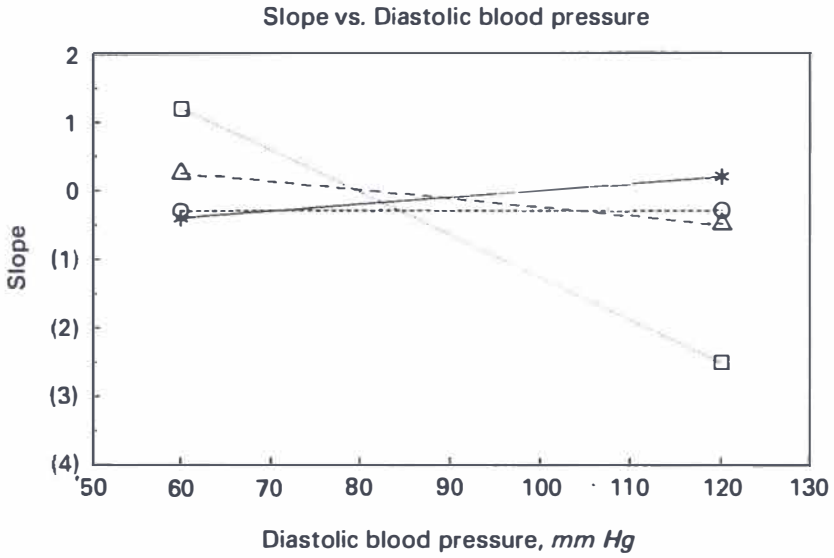

Figure 6 Slope of progression plotted against median diastolic blood pressure over the entire follow-up period. Symbols are: (*) interstitial nephritis. r = 0.81, not significant; (- - ○ - -) glomerulonephritis, r = 0.76. not significant; (- - □ - -) adult polycystic kidney disease. r = -0.88, P< 0.001; (- - Δ - -) others, r = -0.32 not significant. Only regression lines are shown.

To examine whether it is the protein intake as compared to the phosphate intake[83] that is deleterious to the kidney, we plotted the slope of progression against the median urea excretion as well as against median phosphate excretion. Both substances were measured during the entire period of follow-up. No significant correlations were found (slope vs. urea excretion r=-0.41: NS; slope vs. phosphate excretion r=0.38: NS).

The plasma concentrations of essential and semi-essential amino acids are presented in Table 8. Data are shown for the four patient groups as well as the normal values from healthy volunteers.

In general, the plasma concentrations were lower in group A1 than in normal subjects and in group A2 than in group A1. This finding is in good agreement with data published recently, showing that essential and semi-essential amino acids decrease in parallel with the progression of renal disease[84,85].Only phenylalanine, methionine and histidine did not

Table VIII Amino acid profiles (μmol liter).

Amino acids	Normal range N = 100	Group A1 N = 43	B N=39	A2 N = 6	C N = 21
Essential and semi-essential					
Isoleucine	66	58	52	49	49
Leucine	128	113	99	87	88
Valine	231	198	180	180	158
Phenylalanine	58	62	55	55	57
Tryptophan	38	44	38	25	21
Lysine	185	174	166	163	153
Methionine	27	27	24	23	68
Threonine	138	121	114	97	125
Histidine	89	90	87	86	86
Tyrosine	59	53	48	39	40
Non-essential					
Serine	113	100	95	98	109
Glycine	226	262	286	287	336
Alanine	372	380	432	391	412
Ornithine	65	105	107	141	116
Arginine	90	67	63	63	80
Branched chain keto-acids					
KMVA	20	10	11	6	5
KICA	35	18	15	8	9
KIVA	14	10	10	7	7

seem to change with GFR. Without exception, the plasma amino-acid concentrations were lower in group B as compared to group A1. This may be an effect of protein restriction. The data from group C and A2 were in the same range. These findings suggest that the LPD had a greater effect on the essential and semi-essential plasma amino-acid concentrations in mild renal disease. The high methionine concentration in group C may reflect the supplementation of this amino acid.

The results for some non-essential amino acids are also shown in Table 8. In comparison to normal values, the concentrations of glycine, alanine and ornithine were increased in all groups. Serine was rather constant; only arginine was decreased. Similar observations for uraemic patients have been reported by other investigators[86-88]. The plasma branched chain keto-acids in the four experimental groups were markedly decreased in comparison to normal values (Table 8). The decrease in plasma branched chain keto-acids (BCKA's) in group A 1 and group A2 in comparison to values from normal subjects, was even more pronounced than the reduction in the branched chain amino-acids (BCAA's). The plasma BCKA were not different in group A1 versus B or groups A2 versus C.

Discussion

In this prospective, randomized trial on dietary protein restriction in chronic renal insufficiency, we followed our patients for four years. In this period, no harmful effects of the diet were noticed. Patients appeared to be very compliant after a short training period. They stayed compliant even after stopping intensive dietary counseling. Malnutrition did not occur, as measured by body weight and serum albumin, and the amino acid profiles were comparable to those of the patients on a free diet.

Our results after two years of follow-up[52,71] prompted us to evaluate the various diagnostic groups separately. Patients with glomerulonephritis did benefit from the LPD, in contrast to the entire patient population. El Nahas et al[15] found in a small number of patients over a limited period of time that the best results with low protein diets were obtained in patients with tubulointerstitial disease; a marginally positive effect was observed in patients with with glomerulonephritis. Oldrizzi et al[49] found poor results in patients with glomerulonephritis and postulated that in glomerulonephritis the glomeruli are so severely damaged that they are no longer able to hyperfiltrate. The explanation for these discrepant observations is not clear.

No study has shown a beneficial effect of protein restriction in polycystic disease. It is known that in patients with PKD the progression rate depends on the growth rate of the cysts, the number of infections per year and the prevalence of hypertension[89]. As shown in figure 6, we observed that the progression rate in PKD was highly dependent on hypertension. This was not important in the other diagnostic groups. It should be noted that the blood pressure was equally well-controlled in all patient groups and at no time during follow-up there was a significant inter-group difference in blood pressure.

In addition to the dietary protein intake and blood pressure regulation, we investigated the influence of sex on the progression rate. There is an accumulating body of evidence that there are major differences in the progression rate towards ESRD among male and female patients. This has been shown in rats[90,91] and could be retrospectively confirmed in humans[92,93]. In our study, which was randomized for sex, this impression could be reinforced. Male patients from the control groups (A1 and A2) showed a faster decline in renal function. The same male-female differences were observed regardless of whether the patients were analyzed separately according to dietary therapy. However, in our study only the male patients showed an improved slope with dietary therapy, whereas the female patients did not. One observation that may explain this inter-sex difference is that the RNA expression of the renin-angiotensin system is androgen-dependent[91].

In conclusion, after four years of follow-up we are only moderately optimistic about the ability of dietary protein restriction to retard the rate of progression of chronic renal insufficiency. Such dietary therapy should be applied selectively. A high probability of good results can be expected in patients with glomerulonephritis, especially if the patients are male. The rate of progression in patients with adult polycystic kidney disease showed improvement with good blood pressure control. In the other diagnostic groups, blood pressure regulation did not play an important role: this finding is in contrast with other

reports in the literature[94]. Proteinuria was temporarily reduced by the diet and serum phosphorus levels were better controlled.

Patients with chronic renal disease show abnormal plasma amino acid profiles[84-88]. However, no significant differences were seen between the patients given low protein diets and the control groups. This finding, in association with the unchanged body weight, serum total protein and serum albumin, suggests that dietary protein restriction is a safe and effective regimen to delay the progression of renal disease in the above-mentioned selected patient groups.

Chapter 7

Amino-acid profiles in renal patients with and without long-term dietary protein restriction

Summary

Dietary protein restriction to retard the progression of chronic renal insufficiency is highlighted in today's nephrology. Not much is known about its side-effects, e.g. malnutrition. In an attempt to find a useful parameter for nutritional status in these patients, we performed 109 extensive amino-acid (AA)- profiles in a cohort of 247 patients, since 1982 prospectively being followed-up in a trial of dietary protein restriction.

From the data obtained after a median treatment time of 42 months, we conclude that: a) all patients show a typical 'uremic pattern of AA profiles' as described by Kopple et al, b) protein restriction in patients with an initial Glomerular Filtration Rate (GFR) between 31-60 ml/min leads to a more pronounced drop in plasma AA-concentrations than in patients with GFR values below 30 ml/min, c)there exist remarkable intersex differences, and d) taking weight loss as a criterion for malnutrition, no correlations with changes in AA profiles can be found. As branched chain keto acids showed more pronounced reactions to changes in nutritional habits, these acids might be most suitable for the early detection of malnutritive states.

Introduction

Dietary protein restriction to prevent progression of chronic renal failure has gained growing interest in recent years[26-34,52]. Its therapeutic value could be proven in certain diagnosis groups[15,49,95]. Many questions, however, have remained unanswered up to date[18,96]. A major criticism to the diet is that it might cause malnutrition. Although in our Western world an overconsumption of protein exists - over 1.5 g protein/kg body weight per day is not unusual - restricting the intake to a level of 0.4 g/kg per day bears the risk of inducing a negative nitrogen balance[97].

The major problem to evaluate the question of malnutrition arises from the fact that we have no reliable follow-up parameters to assess nutritional status. In common use are anthropometric standards like body mass index, muscle mass and skinfold thickness. However, data derived from these methods are poorly reproducible. Their values depend on the investigator involved and the method used. More objective indicators have been sought in plasma proteins with a short half-life like transferrin, cholinesterase and C_3-

complement. Serum albumin can only serve as a very rough indicator and changes in albumin concentration are usually too late in diagnosing malnutrition.

In the present study we made an attempt to investigate the usefulness of the estimation of plasma amino-acid- as well as their keto-analogue values, for the assessment of nutritional status. Since not much is known about the implications of changes in certain plasma amino-acid concentrations during prolonged protein-restriction in chronic renal failure, and hence no comparisons can be made, the major body of results presented here is kept descriptive and not analytical.

Patients and Methods

All patients visiting the nephrologic outpatient department between January 1, 1982, and April 1, 1984, with a creatinine clearance (CrCl) between 10 and 60 ml/min/1.73 m^2 were considered for a prospective, randomised trial on dietary protein restriction early in the course of chronic renal failure. Excluded were only patients with systemic lupus erythematosus, active vasculitis and Wegener's disease. Patients were stratified according to sex, age (below and above 40 years) and renal function (CrCl below and above 30 ml/min/1.73 m^2). Thereafter, they were randomly allocated to a protein-restricted or a control group.

The quantity of protein prescribed in the restriction groups depended on the degree of renal failure, being 0.6 g/kg/day for patients with a clearance between 31 and 60 ml/min/1.73 m^2 (group B), and 0.4 g/kg/day for patients with a clearance between 10 and 30 ml/min/1.73 m^2 (group C). Their control groups, A1 and A2, respectively, continued their usual diet. At entry all patients visited the dietician for a detailed diet history. Protein-restricted patients were advised to adhere to the above-mentioned diets. All patients received a vitamin/trace element preparation. Patients in group C were supplemented with methionine if necessary, in a dosage of 250 mg t.i.d. orally.

Every three months all patients visited the nephrologic outpatient department. At these occasions many variables were obtained and stored in a computerized data base. Among them were body weight, blood pressure, hemoglobin concentration, hematocrit, platelet count, serum-pH and serum values of creatinine, urea, calcium, phosphorus, bicarbonate, alkaline phoshatase, total protein, albumin, cholesterol and triglycerides. In 24-hour urine specimens the concentrations of urea, creatinine, protein, sodium, calcium and phosphorus were measured. Patients from groups B and C visited the dietician (the same person during the entire study) every 3 months. Patients from the control groups visited her only on indication.

Patients from the control groups were protein-restricted if serum urea concentrations exceeded values of 25 mmol/l. Dialysis was instituted if creatinine clearance dropped below 4 ml/min/1.73 m^2. Some patients received a cadaveric kidney transplant; never because their creatinine clearance had fallen below 4 ml/min, but because a DR-identical kidney became available.

Thus, the 247 patients of this study were divided into four groups:

A1: (n=77)CrCl 31-60 ml/min; no protein restriction,
B: (n=74)CrCl 31-60 ml/min; 0.6 g protein/kg/day;
A2: (n=40)CrCl 10-30 ml/min; no protein restriction,
C: (n=56)CrCl 10-30 ml/min; 0.4 g protein/kg/day.

Outlines of the diet

The diet history was very detailed and included estimations of quantities of energy, protein, sodium, phosphorus, fluid and sometimes potassium. Because protein restriction can have an impact on energy intake, we adapted the diet in case of changes in body weight that were thought to be due to a low energy supply. Thus, the mean energy intake in all groups remained roughly 150 kJ per kg body weight. The protein content of the diet was composed of high biological value proteins. In young patients with higher demands of energy, this sometimes gave troubles. Especially in the strongest-restricted patients, sometimes the advice to eat low-protein bread was given. Calculation of amino acid intake in strongly restricted patients often showed methionine deficiency. The above-mentioned methionine supplementation then was given.

With protein restriction a phosphorus restriction is concomitant. In group C phosphorus intake amounted to 500- 800 mg/day.

The dietician supplied the patients with a list of the protein content of usual all-day food. A variation list was added, as well as a list with products for which no limitation in intake was necessary.

At each follow-up visit the patients were instructed how to go on with the diet. At the first visit after 3 months, some patients in the protein-restricted groups had lost weight. Therefore, these patients were supplied with 500-800kJ more than calculated from the initial energy demands. Patients eating low-protein bread sometimes developed a strong aversion against this product. If so, they received a new diet in which normal bread was supplied but with extra restriction of other proteins, especially from animal sources.

From the urinary urea excretion at the previous visit the protein intake was calculated. The dietician was informed about the result and used it to motivate the patient to continue the advised diet.

After a median period of 42 months of follow-up from 109 randomly chosen patients, a blood sample was drawn for extensive amino-acid profile analysis.

This was performed by a modified Stein-Moore method using cation exchange chromatography on the analyzer LC 5001 (Biotronik, Puchheim, FRG). Separations were made in Li-citrate buffer using high performance columns with a 7 µm resin. Keto acids were analysed by a modification of the HPLC-method of Kieber[98]. After removal of amino acids by mixed ion exchange systems, derivatisation with o-phenylenediamine follow-

ed. We were thus able to do large sample numbers (transforming the substances to be measured to quinoxalinole derivates) automatically by HPLC without any interferences.

Statistical analysis was performed by means of the PC-Statistical Analysis System (SAS)[99]. Data are presented as mean values ± standard deviation. Statistical differences were disclosed by means of a two-tailed Student-T test. Normal plasma values of the various amino-acids were derived from a group of 100 normal healthy volunteers (50 male, 50 female). These normal values appeared to be in accordance with those elsewhere published, derived from large groups of healthy individuals[100].

Results

248 patients were enrolled in the trial between January 1, 1982 and April 1, 1984. The outcome regarding the effect of dietary protein restriction on the progression rate of chro-

Table I Comparison between protein-restricted and control groups with respect to plasma amino-acid concentrations.

Amino acids	Normal range (n=75)	A1 (n=43)	B (n=39)	A2 (n=6)	C (n=21)
Ess. and semi-ess.					
Isoleucine	66 ±13	61 ± 17	56 ± 17	58 ± 25	57 ± 22
Leucine	127 ±22	118 ± 34	106 ± 31	107 ± 42	95 ± 27
Valine	231 ±41	202 ± 42	186 ± 40	195 ± 61	192 ±123
Phenylalanine	58 ±11	63 ± 14*	56 ± 12	53 ± 11	57 ± 13
Tryptophan	38 ±18	45 ± 15	39 ± 13	36 ± 21	36 ± 33
Lysine	185 ±37	176 ± 37	164 ± 32	171 ± 46	161 ± 35
Methionine	27 ± 5	33 ± 24	30 ± 27	23 ± 3*	98 ± 63
Threonine	138 ±34	126 ± 29*	112 ± 23	100 ± 19*	127 ± 30
Histidine	89 ±16	92 ± 16	87 ± 13	87 ± 11	92 ± 18
Tyrosine	58 ±14	58 ± 22*	49 ± 17	44 ± 16	46 ± 14
Non-ess.					
Serine	122 ±23	104 ± 22*	95 ± 18	89 ± 24	107 ± 27
Glycine	226 ±56	298 ± 92	296 ± 80	361 ±198	353 ±124
Alanine	372 ±92	390 ±101*	447 ±122	420 ±161	466 ±151
Ornithine	65 ±21	109 ± 24	107 ± 24	129 ± 23	118 ± 21
Arginine	90 ±23	66 ± 23	62 ± 23	62 ± 11	73 ± 20
BCKA					
KMVA	20 ± 6	11 ± 4	10 ± 4	10 ± 6	7 ± 3
KICA	35 ±11	19 ± 8*	15 ± 6	14 ± 14	10 ± 5
KIVA	14 ± 4	12 ± 5	11 ± 5	10 ± 10	8 ± 7

* $p < 0.05$ if compared to Pr-restricted group.

nic renal failure has been discussed elsewhere[52,95]. In first instance, we studied the means of plasma amino-acid levels in the four patient groups (Table I). It can be seen clearly, that for essential amino-acids in general there was a continuous decline in concentration from normal subjects to group A1 and further on to group A2. This result is in good agreement with data published recently elsewhere, showing that essential and semi-essential amino acids are decreasing in parallel to the progression of renal disease[101,102]. Only the concentrations of phenylalanine, methionine and histidine were rather constant.

Without any exception a drop in concentration was found from group A1 to group B, reflecting the consequence of the low-protein diet (LPD) directly. The data of group A2 and C were in the same range. Obviously, LPD pronounced influencing the essential and

Table II Comparison of plasma amino-acid concentrations in male vs. female patients.

Amino acids	Normal range males	males (n=54)	Normal ranges females	females (n=55)
Ess. and semi-ess.				
Isoleucine	74 ± 17	66 ± 18[a]	60 ± 10	51 ± 16
Leucine	142 ± 23	119 ± 33[b]	116 ± 21	99 ± 30
Valine	250 ± 38	210 ± 80[c]	218 ± 44	178 ± 39
Phenylalanine	61 ± 13	60 ± 14	55 ± 10	58 ± 12
Tryptophan	42 ± 20	42 ± 17	39 ± 16	39 ± 23
Lysine	190 ± 37	173 ± 38	185 ± 37	165 ± 33
Methionine	28 ± 5	39 ± 36	26 ± 5	47 ± 49
Threonine	131 ± 27	123 ± 30	146 ± 40	116 ± 25
Histidine	88 ± 14	94 ± 16[d]	90 ± 18	87 ± 13
Tyrosine	63 ± 13	50 ± 20	56 ± 14	53 ± 19
Non-ess.				
Serine	107 ± 20	97 ± 22	119 ± 25	104 ± 22
Glycine	244 ± 50	293 ± 96	228 ± 61	329 ±109
Alanine	386 ± 93	453 ±139[d]	368 ± 92	402 ±104
Ornithine	73 ± 22	113 ± 25	61 ± 19	109 ± 23
Arginine	94 ± 23	66 ± 23	86 ± 22	65 ± 21
BCKA				
KMVA	21 ± 7	11 ± 4[e]	19 ± 6	9 ± 3
KICA	36 ± 11	18 ± 9[e]	38 ± 12	13 ± 6
KIVA	15 ± 5	13 ± 6[e]	15 ± 4[a]	8 ± 4

[a] $p<0.001$ if compared to female.
[b] $p<0.005$ if compared to female.
[c] $p<0.01$ if compared to female.
[d] $p<0.05$ if compared to female.
[e] $p<0.0005$ if compared to female.

semi-essential plasma amino-acid concentrations in already mild renal disease. The high methionine concentration in group C was due to the supplementation with this amino-acid. Regarding the non-essential amino-acids, in comparison to normal values the concentrations of glycine, alanine and ornithine were raised in all groups. Serine was rather constant and only arginine was decreased.

The branched chain keto-acids (BCKA, e.g KMVA=α-keto-ß-methyl-n-valeric acid; KICA= α-ketoisocaproic acid and KIVA= α-ketoisovaleric acid) of the four investigated groups were markedly decreased in comparison to normal individuals. Even more impressive than for branched chain amino acids,the decline in plasma BCKA's from normal subjects to group A1 and to group A2 illustrates the progression of the disease. The BCKA-data of the corresponding groups with and without LPD were in the same range.

In Table II a breakdown by gender is presented. Normal values, obtained from 39 healthy men and 36 women are given as reference.

Table III Comparison of plasma amino-acid concentrations in patients above or below 2 kilograms weight loss over a median period of 42 months.

	more than 2 kg weight loss (n=25)	no weight loss (n=84)
Ess. and semi-ess.		
Isoleucine	56 ± 16	58 ± 18
Leucine	108 ± 28	108 ± 34
Valine	184 ± 35	195 ± 71
Phenylalanine	58 ± 10	59 ± 14
Tryptophan	35 ± 12	41 ± 22
Lysine	165 ± 30	169 ± 38
Methionine	48 ± 51	42 ± 40
Threonine	113 ± 24	121 ± 28
Histidine	88 ± 15	91 ± 15
Tyrosine	49 ± 15	52 ± 20
Non-ess.		
Serine	100 ± 21	100 ± 22
Glycine	294 ± 72	315 ±111
Alanine	433 ±103	424 ±132
Ornithine	113 ± 20	109 ± 25
Arginine	69 ± 23	64 ± 22
BCKA		
KMVA	10 ± 4	10 ± 4
KICA	16 ± 7	15 ± 8
KIVA	11 ± 5	11 ± 6

In the essential- and semi-essential amino-acid concentrations striking intersex differences were obtained for isoleucine, leucine, valine as well as histidine. As non-essential amino-acid, only alanine was slightly higher in males than in females. All BCKA showed marked intersex differences.

Searching for a parameter representing status of nutrition, we only had body weight and serum albumin available. Since serum albumin in our patients did not change except in a few patients with the nephrotic syndrome, statistics with this variable was not possible. Therefore, we had to perform our calculations with body weight. A consistent loss of more than 2 kg after 36 months of follow-up if compared to trial-entry was used to create two groups. Thus, 41 patients with consistent weight loss of more than 2 kg were found. Mean weight loss in these patients was 4.2 kg (range 2-12 kg, SD: 2.2 kg). From 25 of them we had plasma amino-acid profiles available. Fifteen of them belonged to the protein- restricted patients, from which again nine from group C, i.e. the most stringently restricted patients.

As is shown in Table III, no significant differences in plasma amino-acid levels between 'weight-losers' and patients with constant or gaining weight were found. Hence, there was no relation between the loss of lean body mass and a change in plasma amino-acid profiles.

Discussion

A number of alterations in plasma levels of amino-acids has been described in chronic renal disease. Deviations are often similar to those seen in protein malnutrition, but some changes appear to be caused by uremia per se[103,104]. Thus, elevated plasma levels of most of the non-essential amino-acids with the exception of serine are characteristic, resulting in decreased valine/glycine and serine/glycine ratios[104]. Kopple et al[105] reported decreased overall plasma levels and lower plasma tyrosine/phenylalanine ratios in chronic uremic patients, the latter possibly caused by hydroxylase impairment in enzymatic renal conversion of phenylalanine to tyrosine. This observation is in good accordance with our findings where a decrease in plasma tyrosine/phenylalanine ratio in relation to a lower GFR was observed.

Whether this phenomenon is also true in Kwashiorkor, where it has been described, remains still controversial[105].

Diminished concentrations of the BCAA's leucine and isoleucine may be secondary to malnutrition, as those of tryptophane, histidine and lysine. In our plasma amino-acid analysis, no changes in these amino acids were established (Table I). A decrease in valine, however, might be more related to uremia[103,106]. Again, we found no statistically significant change.

Beside the evaluation of plasma AA, we made in the present study an attempt to elucidate possible responses from the plasma keto-analogues in terms of underlying disease and dietary protein intake. Until some years ago, there was only little information availa-

ble for interpretation of both plasma fractions, e.g. AA profiles and their corresponding α-keto-acids[107]. Thus, in our study, according to former reports[105,108], a decline of all three BCKA even in mild chronic insufficiency without any clinical signs of malnutrition could be demonstrated, which was more pronounced than the change in plasma AA levels. We, therefore, state that BCKA are more sensitive parameters than the corresponding AA with regard to metabolic dysfunction. Future studies will enable us to learn more about the key role of plasma KA and their corresponding AA in all kinds of diseases.

Chapter 8

The impact of gender on the progression rate of chronic renal insufficiency and the responsiveness to low-protein diets

Summary

Dietary protein restriction to prevent the progression of chronic renal insufficiency has become one of the major initial therapeutic modalities in nephrology. The target group for this treatment remains poorly defined. In a 247-patient one center randomized, prospective trial over 4 years we could show that beneficial effects are exclusively to be expected in patients with chronic glomerulonephritis. From our data we derive strong indication that the diet is only useful in male patients. There appear to be tremendous intersex differences in the progression rate of chronic renal failure. The causes of the found differences, backed up by animal experiments, remain in humans speculative. The here reported findings may further optimize the treatment with low protein diet by restricting it in those patients where a benefit can be expected.

Introduction

In recent years protein-restricted diets to prevent the progression of chronic renal insufficiency have gained a well-deserved place in the treatment of nephrologic patients. This results from ideas that were postulated at the beginning of this century[51], but disappeared in the background once renal replacement therapy became available. The comeback of the conservative way of treatment was paved by several non-randomised or retrospective[26-34], and one randomised, prospective trial[52,53,71,95]. From foregoing trials, the right target groups are increasingly better defined and hence the diet can be applied more accurately in diagnosis groups where benefit is likely, e.g. chronic glomerulonephritis in contrast to adult polycystic kidney disease and hypertensive nephrosclerosis[15,49,95].

Animal experiments have shown that male rats respond differently to renal ablation than female animals[90,108]. Male rats have a more rapid decline towards end-stage renal failure and die earlier in uremia. Although in several rat models a beneficial effect of a low protein diet on the progression rate of renal failure could be established[22,109], the relationship between gender and the response to low protein diets was not yet subject of study. The situation in humans is even more obscure, although there are a few indications that gender might be a prognostic factor in renal disease[93,110].

The validation of trials on dietary protein restriction is hampered by statistical problems[82]. In man reliable results can only be derived from prospective, randomised trials in large numbers of patients. In the 'Groningen-trial'[52,71,95], 247 patients are prospectively followed since 1982. Since it is the only large scale trial that is stratified for sex, age and initial degree of renal failure, these patients form a good cohort to study the effect of gender on the progression rate of chronic renal insufficiency and to assess its impact on the responsiveness to low-protein diets.

Patients-Methods

All patients visiting the nephrologic outpatient department between January 1, 1982, and April 1, 1984, with a creatinine clearance (CrCl) between 10 and 60 ml/min/1.73 m^2 were considered for a prospective, randomised trial on the use of protein-restricted diets early in the course of chronic renal insufficiency. Excluded were only patients with systemic lupus erythematosus, active vasculitis and Wegener's disease. Patients were stratified according to sex, age (below and above 40 years) and renal function (CrCl below and above 30 ml/min/1.73 m^2). Thereafter, they were randomly allocated to a protein-restricted or a control group.

The quantity of protein prescribed in the restriction groups depended on the degree of renal failure, being 0.6 g/kg/day for patients with a clearance between 31 and 60 ml/min/1.73 m^2 (group B), and 0.4 g/kg/day for patients with a clearance between 10 and 30 ml/min/1.73 m^2 (group C). Their control groups, called A1 and A2, respectively, continued their usual diet.At entry all patients were sent to the dietician for a detailed diet history. Protein-restricted patients were advised to adhere to the above-mentioned diets. All patients received a vitamin/trace element preparation. Patients in group C were supplemented with methionine if necessary, in a dosage of 250 mg t.i.d. orally.

Every three months the patients visited the nephrologic outpatient department. At these occasions many variables were obtained and stored in a computerized data base. Among them were body weight (BW), blood pressure, hemoglobin concentration, hematocrit, platelet count, serum-pH and serum values of creatinine, urea, calcium, phosphorus, bicarbonate, alkaline phoshatase, total protein, albumin, cholesterol and triglycerides. In 24-hour urine specimens the concentrations of urea, creatinine, protein, sodium, calcium and phosphorus were measured. Patients from groups B and C visited the dietician (the same person during the entire study) every 3 months. Patients from the control groups visited her only on indication. Patients from the control groups were protein-restricted if serum urea concentration exceeded values of 25 mmol/l. Dialysis was instituted if creatinine clearance dropped below 4 ml/min/1.73 m^2. Some patients received a cadaveric kidney transplant; never because their creatinine clearance had fallen below 4 ml/min, but because a DR-identical kidney became available.

Outlines of the diet

The diet history was very detailed and included estimations of quantities of energy, protein, sodium, phosphorus, fluid and sometimes potassium. Because protein restriction can have an impact on energy intake, we adapted the diet in case of changes in body weight that were thought to be due to a low energy supply. Thus, the mean energy intake in all groups remained roughly 150 kJ per kg body weight.

The protein content of the diet was composed of proteins of high biological value. In young patients with higher demands of energy, this sometimes gave troubles. Especially if they were from the group of patients with the strongest restriction, e.g. 0.4 g/kg BW, sometimes the advice to eat low-protein bread was given.

Calculation of amino acid intake in strongly restricted patients often showed methionine deficiency. The above-mentioned methionine supplementation was given here.

With protein restriction a phosphorus restriction is concomitant. In group C phosphorus intake amounted to 500- 800 mg/day.

The dietician supplied the patients with a list of the protein content of usual all-day food. A variation list was added, as well as a list with products for which no limitation in intake was necessary.

At each follow-up visit, the patients were instructed how to go on with the diet. At the first visit after 3 months, some patients in the protein-restricted groups had lost weight. Therefore, these patients were supplied with 500-800kJ more than calculated from the initial energy demands.

Patients eating low-protein bread sometimes developed a strong aversion against this product. If so, they received a new diet in which normal bread was supplied, but with extra restriction of other proteins, especially from animal sources. From the urinary urea excretion at the previous visit the protein intake was calculated. The dietician was informed about the result and used it to motivate the patient to continue the advised diet.
Thus, four patient groups were created:

A1: (n=77),CrCl 31-60 ml/min; no diet,
A2: (n=40),CrCl 10-30 ml/min; no diet,
B : (n=74),CrCl 31-60 ml/min; 0.6 g protein/kg BW/day,
C : (n=56),CrCl 10-30 ml/min; 0.4 g protein/kg BW/day.

Results

Two hundred and forty-seven patients were included in the study before April 1, 1984. The general effect of the diet and the impact of the several diagnosis groups have been published elsewhere[71,95]. Sex distribution was equal in all 4 groups: A1: 41 males, 36 females; B: 43 males, 31 females; A2: 23 males, 17 females, and C: 25 males , 31 females.

So, over the entire population 68 from a total of 132 males were protein-restricted against 62 from 115 females.

To study the intersex-differences on the progression of renal failure and the reponse to protein restriction, several statistical modalities can be used. Ultimately, the most important parameter is how many patients progress to end stage renal failure in the sense of dialysis-dependency or death due to renal causes. This is depicted in Table I. From this table it becomes clear that in the *control groups* more males became dialysis-dependent after 4 years of follow-up than females. Due to the low number of thus lost patients, however, it did not reach significance. The table also shows that the response to *protein restriction* in female patients seemed to be negligible; protein-restricted women even tended to be more prone to become dialysis-dependent.

Table I Number of patients entering dialysis, split-up by sex and dietary advice.

		dialysis-dependent
male	Pr-restr (N=68)	6
	controls (N=64)	15
female	Pr-restr (N=62)	8
	controls (N=53)	5

(Chi-square analysis revealed no significant differences between males and females entering dialysis (intra-sex differences) if compared to the total population, but significantly less males on protein-restriction entered dialysis if compared to females: x2 (Yates' correction) = 5.09, df = 1; $p < 0.05$).

Another reliable method to assess dietary effects is to compare the slopes of creatinine clearance against time from every individual before and after inclusion in the trial. From 149 patients we had creatinine clearance values of at least 9 months before enrolment in the study. The intersex-differences of these 149 patients are provided in Table II. We see a statistically significant benefit from the diet for male patients ($p<0.01$). This table reinforces the aforementioned impression from Table I that the spontaneous rate of decline in renal function in male patients is significantly higher than in females.

To elucidate whether the differences were explained by dietary compliance, we estimated the dietary protein intake by means of 24 h urinary urea excretion values. In general we observed a very good dietary compliance[52,71], which is in contrast to the common opinion about compliance to low protein diets. The dietary compliance as assessed by the measurement of 24 h urea excretion is depicted in figure 1. Although male patients started with a significant higher urea excretion, they reduced their protein intake to about the same levels as female patients did and remained compliant. In the control groups no change in protein intake occurred.

Another factor that might play a role in the found differences is the higher incidence of glomerulonephritis in our male patients (47 males/ 33 females), a disease that is known to be correlated with a good response to low protein diets[49,95]. Comparing all available slopes after inclusion, the results, provided in Table III were found. Relating it to Table II, it can be concluded that progression of glomerulonephritis-induced renal failure is more

Table II Median slopes of decline in creatinine clearance before and after inclusion in the trial.

		slope before	p-value	slope after	n
Male	Pr-restr	-0.34	0.01	-0.18	41
	(p-value) *	(N.S.)		(0.05) *	
	controls	-0.29	N.S.	-0.27	42
Female	Pr-restr	-0.22	N.S.	-0.14	36
	(p-value)	(0.05)		(N.S.)	
	controls *	-0.19	N.S.	-0.10 *	32

* $p<0.05$.

(For calculations of intra-individual slope differences, Wilcoxon rank sum test and for interg-roup differences Mann Whtiney-U test were used).

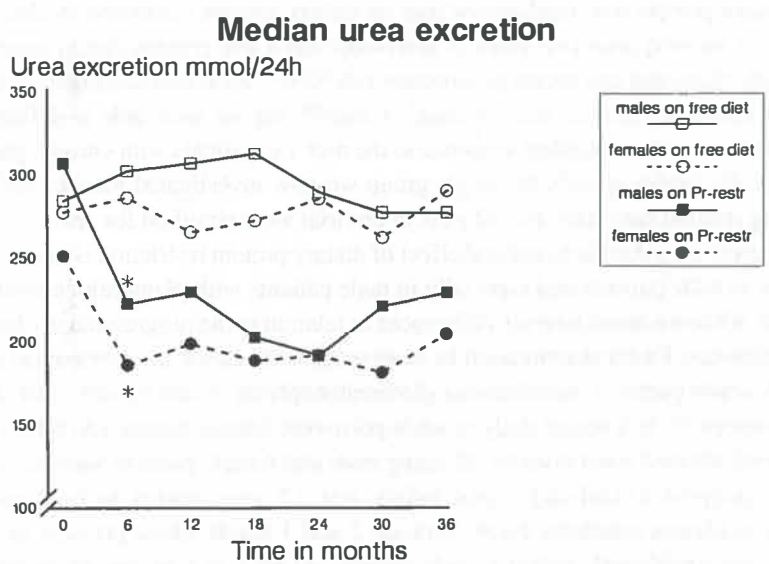

Figure 1 Median 24 h urinary urea excretion of patients followed for at least 36 months, split-up by sex and dietary advice. [*: comparison urea excretion between t = 0, and t = 6 months: $p< 0.001$ (Wilcoxon signed rank test). At every moment during follow-up, p-values for differences between urea excretion on free diet versus pr-restricted diets were<0.001 (Mann Whitney U-test)].

rapid than of all other subgroups. Protein restriction caused a less steep slope in males (-0.27 against -0.38), whereas in females with glomerulonephritis dietary protein restriction seemed to accelerate the downhill course of renal function (-0.36 against -0.15). A statistical artefact, caused by the unequal distribution of dietary advice in glomerulonephritic women, however, can not be excluded.

Table III Median slopes of decline in creatinine clearance in patients with Glomerulonephritis.

Sex	n	diet	slope	p-value
Male	22	yes	-0.27	<0.05
	25	no	-0.38	
Female	12	yes	-0.36	<0.01
	21	no	-0.15	

Discussion

In this large prospective, randomised trial on dietary protein restriction in chronic renal failure, we showed after two years of follow-up that a low protein diet in general has a beneficial effect and can retard progression rate[52,53,71]. Renewed calculations after four years of follow-up showed less optimistic results[95], but we were able to define a target group that showed an excellent response to the diet: i.e. patients with chronic glomerulonephritis. To further specify the target group we now investigated intersex differences, obtaining reliable data since the subjects in our trial were stratified for sex.

We report here that the beneficial effect of dietary protein restriction is due to the good response in male patients and especially in male patients with glomerulonephritis.

Little is known about intersex differences in relation to the progression of chronic renal insufficiency. Faster deterioration in male patients is known in several renal diseases, e.g. IgA-nephropathy[111], membranous glomerulonephritis[112] and patients with the nephrotic syndrome[93]. In a recent study in adult polycystic kidney disease (ADPKD), a genetically well-defined renal disorder affecting male and female patients with the same probability, progress to end-stage renal failure was 7,2 years earlier in male patients[110]. Already at plasma creatinine levels between 2 and 3 mg/dl, blood pressure in ADPKD patients was significantly higher in male patients. ADPKD is a disease where blood pressure regulation correlates stronger than other diseases with progression of renal disease[95]. Interestingly, in prepubertal renal diseases, e.g. nephronophtisis or cystinosis, male and female patients progress with the same velocity to terminal renal failure[110].

The backgrounds of the findings reported here remain obscure. The main question arising is: why do women, especially those with chronic glomerulonephritis, show such a deviant response to the diet? Several hypothetical explanations can be given.

Firstly, since males have a more rapid decline towards end-stage renal disease, a positive effect of the diet may at first instance become visible in this group.

Secondly, the relatively higher number of male patients with glomerulonephritis, if compared to females, may, at least partially cause the overall better response in males since glomerulonephritis apparently is the best target group for the diet.

Thirdly, we found in our male patients a more pronounced drop in initial urea excretion after installing the diet. It cannot be excluded that the relatively more pronounced protein restriction in males, thus confirmed, is responsible for the found differences.

Other assumptions are based on interesting findings in animal experiments, where intersex differences in progression to end-stage renal failure are partially clarified.

Remuzzi et al[90] found that male Munich Wistar rats developed spontaneously proteinuria, accompanied by higher single-nephron glomerular filtration rate (SNGFR) and elevation of the ultrafiltration coefficient (K_f). There was no intersex difference regarding to the total number of glomeruli and the glomerular size. The authors speculated that the defects in the glomerular basement membrane caused by increased hydraulic pressure, are genetically determined.

Elema and Arends[113] described more severe proteinuria and focal/segmental glomerular hyalinosis and sclerosis in male ageing Wistar rats. Gender-related differences in the development of glomerular injury were also reported after renal ablation[114], and conversely castration has been noted to attenuate progressive glomerular injury in this model[115].

Several hypotheses can be proposed by which renal damage may be aggravated by sex-hormone dependent factors. It is known that estrogens and androgens modify the activity of the renal angiotensin system[116]. The recent demonstration that testosterone increases messenger RNA for various components of the renin system within the kidney[117] and extrarenal tissues[118] may point to a significant sex hormone-dependent effector mechanism. Another animal study concludes that exogenous testosterone administered after unilateral nephrectomy, has different effects on compensatory renal hypertrophy (CRH) in male versus female rats[108]. Where testosterone had no significant effect in true male or in pseudo-hermaphrodite male animals, female rats responded with a clearly increased CRH. The concomitant increase in glomerular hyperfiltration then may lead to progressive renal damage. Recently, Blantz et al[91] treated ovariectomized female rats with androgen administration over 16 weeks, After 6 and 16 weeks, micropuncture studies were performed. SNGFR as well as renal size rose in comparison to untreated, ovariectomized controls. The maximum SNGFR was reached after 6 weeks, whereafter it remained in the hyperfiltration-range. Renal size increased further, primarily tubular in origin. Blood pressure did not change, excluding a systemic haemodynamic factor to be the main cause.

In summary, protein-restricted diets have a, -probably clinically important- sex-dependent action. Large clinical trials in many patients will elucidate whether our finding can be confirmed after longer periods of follow-up.

Chapter 9: General discussion

Dietary protein restriction in chronic renal failure: an update

Summary

In recent years, the prescription of dietary protein restriction (DPR) in order to slow down the progression rate of chronic renal disease has been a major field in nephrology. In this update a brief historical overview is given, as well as a critical review regarding the now available data from clinical trials. Furthermore, the theoretical backgrounds of DPR are discussed. It is concluded that DPR has profound effects on the kidney. In certain diseases, especially primary glomerular diasease, the beneficial effect seems proven. However, many questions remain to be answered, e.g. how to apply reliable statistics in chronic renal failure, how to obtain and monitor patient compliance, and the definition of the exact target group since the diet has a selective effect.

Most of these questions will be answered in the coming years when data become available from large scale multicenter trials.

Awaiting these results, proposals for the treatment with diet are made, based on the facts that are now to our knowledge.

Introduction

Already since the turn of the century protein-restricted diets have been advocated to relieve uremic symptoms in patients with advanced chronic renal insufficiency. Volhard[51] used a 20-30 g protein diet and concluded as early as 1918 that this intervention was also able to postpone the increase in the serum urea concentration and hence to prolong the patient's life. By that time the main problem in the use of these diets was the concurrent negative nitrogen balance, leading to protein malnutrition syndromes. It was in 1964 that Giovannetti and Maggiore[119] established an equilibrium in this balance by using diets as low as 18 grams of protein per day, supplemented by essential amino acids (EEA), shortly thereafter followed by the well-known potato-egg diet[120].

The main purpose of these diets was to improve life quality by lowering serum urea. The indication: retardation of the progression rate of renal disease, firstly stated by Volhard and in 1939 reappraised by animal experiments of Farr and Smadel[19], disappeared in the background since in the sixties the tremendous prospects of dialysis and transplantation became prominent. Renal replacement therapy, however, is very expensive. Cuts in medical budgets originated a change in attitude in favour of more conservative ways of treatment.

It was in 1972 that Kluthe[25] demonstrated once more that DPR deserved its place in the treatment of chronic renal failure: in a large series of chronic uremics who had been following the potato-egg diet, containing 20-25 g protein daily, the survival was much longer compared to patients on a 40 g protein diet.

The conclusions reached so far led to the concept, that if DPR would be instituted early in the course of chronic renal failure the need for renal replacement therapy could be omitted or at least postponed. It was Giordano[17] who showed in 1981 in a limited number of patients that DPR instituted at serum creatinine levels between 2.0 and 3.0 mg/dl improved survival time (to dialysis or transplantation) to 3.8 years, compared to a matched control group on a normal protein intake with a survival time of only 8 months.

Since then several retrospective and prospective trials have confirmed a beneficial effect of a low-protein diet on the progression of renal insufficiency[26-34].

Problems in designing and conducting DPR-Trials

In validating the results of DPR trials, there are two major problems: first how to monitor the progression rate of renal insufficiency and secondly the way how statistics should be applied[55,82]. Most trials used the reciprocal of the serum creatinine value as Glomerular Filtration Rate (GFR) marker as was advocated by Mitch and Walser[56]. This method, however, has several disadvantages. Firstly, it is well known that there exists tubular secretion of creatinine, becoming more important once renal failure progresses[59,150]. Secondly, the serum creatinine value itself is very dependent on the quantity of meat ingested. This leads us to the creatinine clearance, which is neither a true GFR marker[149]. Shemesh et al[68] compared it with alternative clearance methods and found bad correlations (Figure 1). Radio isotope clearances, probably the most reliable method, are less suitable in clinical trials since their repeated measurements may lead to an unacceptable load of radioactivity.

The ultimate parameter for establishing the effect of an intervention like DPR is how many patients progress to end-stage renal disease (dialysis or death due to renal causes). This, however, requires a long period of follow-up. In our trial for example, we found that the results after 2 years were quite different from those obtained after 4 years.

In the extension of the 'Groningen Trial', as described in Chapter 6, four statistical modalities to assess the effect of the diet were used:

1) how many patients progressed to end-stage renal disease?
2) how were the differences in the slope of change of creatinine clearance against time in DPR patients compared to their controls?
3) how did this slope change before and after institution of the diet?
4) how was survival, taking a 50% loss of GFR as the non-survival criterion?

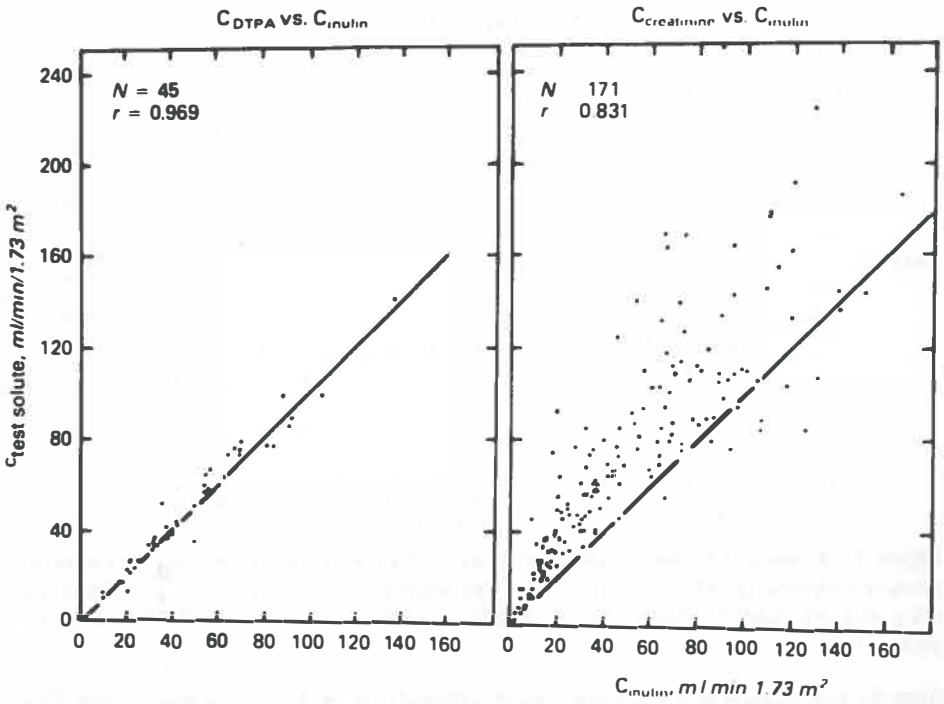

Figure 1 right panel: creatinine versus inulin clearance in a glomerulopathic population (n = 171), left panel: DTPA clearance versus simultaneous inulin clearance in 43 glomerulopathic patients. Dashed lines represent local laboratory normal values. (Reprinted by permission of Kidney International).

Results from the 'Groningen-trial'

In 1984, we reported a general beneficial effect of the diet on the downhill course of chronic renal failure[52]. This trial in which 247 patients participate runs since 1982. 129 patients were randomly assigned to DPR (0.4-0.6 g/kg/day) and 118 to a control group, continuing their usual dietary habits. Stratification took place according to age, sex and degree of renal function. Patients on the diet visited the dietician every 3 months during the first 24 months of the trial, thereafter, like controls, only on indication.

Urea excretion dropped significantly in DPR patients as a sign of good dietary compliance and remained low, even without frequent visits to the dietician. Biochemical parameters, as well as anthropometric data showed no signs of malnutrition. Amino-acid profiles were identical in controls and in DPR patients. Proteinuria decreased significantly.

On the progression rate, the diet appeared to have a selective effect: only patients with primary glomerular disease, especially patients with glomerulonephritis, responded to the diet (fig. 2). Over the entire patient population the effectiveness of the diet depended on the statistical modality used, where the most important indication for effectiveness comes

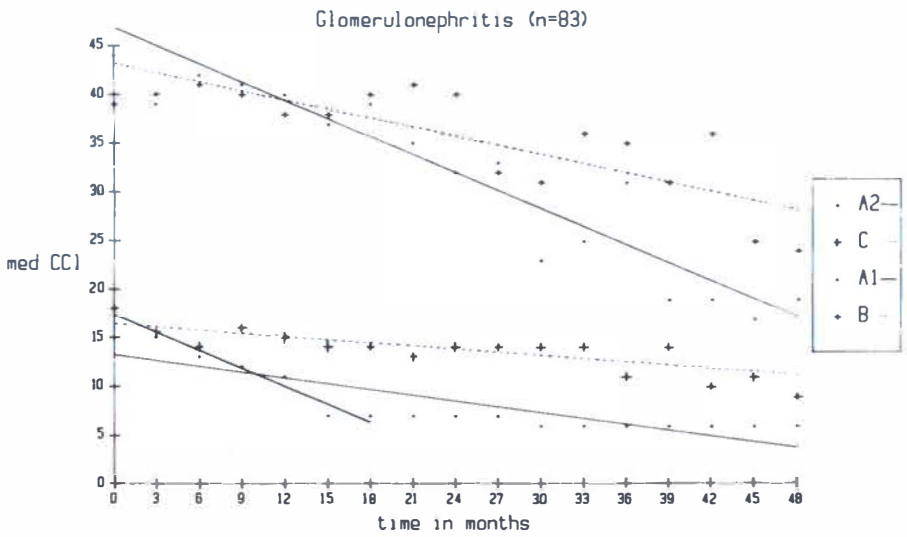

Figure 2 Relation of median creatinine clearance (CCL) with time in months for patients with glomerulonephritis (n=83). A1: initial CCL 31-60 ml/min: no diet; B: initial CCL 31-60 ml/min; 0.6 g protein/kg/day; A2: initial CCL 10-30 ml/min; no diet; C: initial CCL 10-30 ml/min; 0.4 g protein/ kg/day.

from the comparison how many patients progressed to ESRD. In the control group, 25 patients became dialysis dependent, against only 14 in the DPR group. Survival statistics, using the 50% GFR-loss criterion showed exclusively significant benefit for patients with low initial GFR, whereas linear regression on creatinine clearance over time showed no benefit at all over the entire patient population (fig 3).

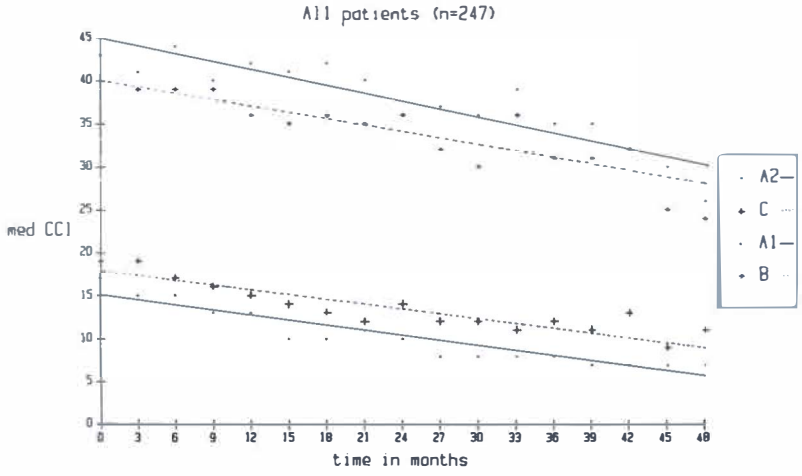

Figure 3 Relation of median creatinine clearance (CCL) with time in months for the entire patient population (n=247). For labels see Fig. 2.

Using patients as their own control, comparing slopes of change in creatinine clearance before and after inclusion in the trial, again a beneficial effect was found in patients with low initial GFR. Striking intersex differences were found. Males showed a more rapid decline towards end-stage renal failure, but responded in a positive way to the diet, whereas female patients could not benefit from the dietary manipulation at all.

Patients with adult polycystic kidney disease depended regarding their progression rate primarily on good blood pressure regulation, whereas in other diagnosis groups this fact played only a minor role.

In conclusion the authors of the 'Groningen trial' are only moderate optimistic about applying the diet in a general nephrological population, whereas excellent results can be expected in the mentioned subgroup with primary glomerular disease. There were no diabetics included in this trial, but studies performed up to now in limited numbers of patients show positive effects[121]. This is in accordance with the Groningen-results, since in diabetic nephropathy the lesions are predominantly glomerular.

Theoretical backgrounds about the mechanism of DPR

Chronic renal failure is characterized by progressive glomerulosclerosis (GS). This is the case in the animal experiment, where some rat strains develop spontaneously GS, as well as in humans with more advanced stages of renal disease. Our understanding of the mechanism that leads to the initiation and progression of GS was recently reviewed by El Nahas[133].

Summarizing the most important factors that can be held responsible for the effects of DPR:

1) alterations in glomerular hemodynamics
2 reduction of 'compensatory renal growth'
3) dietary induced immunological alterations
4) alteration of tubulo-glomerular feedback
5) concomitant reduction of phosphate intake
6) concomitant reduction of lipid intake

Most of the above-mentioned possible mediators are wrapped together in Figure 4.

ad 1) Hemodynamical alterations. A most challenging concept, trying to explain the progression of renal disease was postulated by Brenner in 1982[50]. Once the total amount of nephrons underscores a critical limit, the remaining nephrons are hyperperfused to warrant a normal kidney function. Although unnoticed by the clinician, these hyperperfused and hence hyperfiltrating nephrons, will loose their function, due to a sustaining over-pressure on the glomerular capillary wall and mesangium.

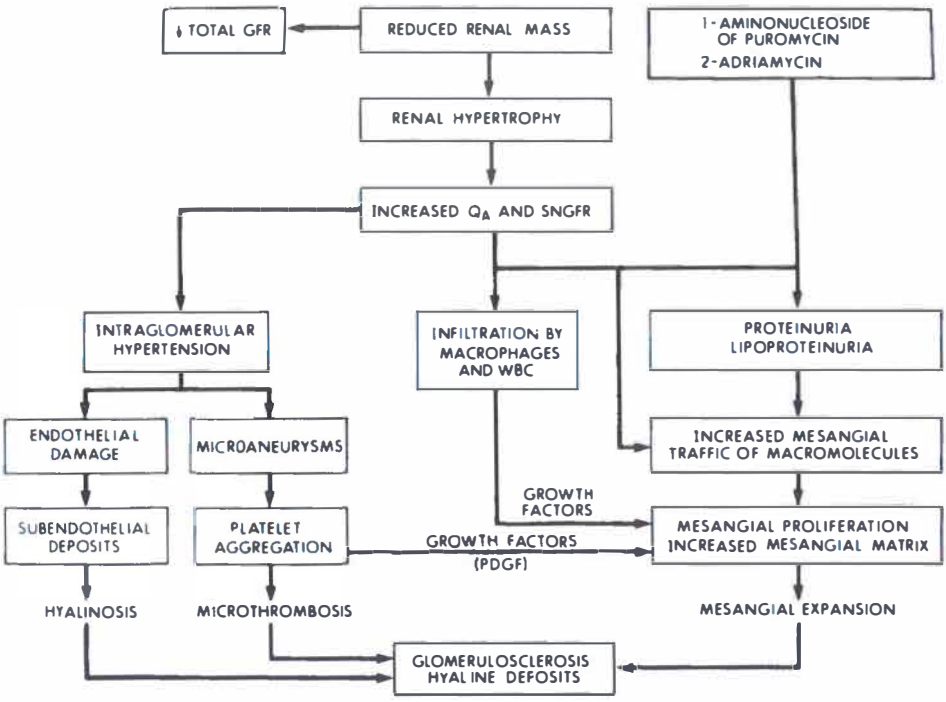

Figure 4 Summary of factors that may lead to the final common pathway 'glomerulosclerosis'. (Reprinted by permission from 'Nephrology', Proc. of the Xth Int. Soc. Nephr., London, 1987. Publ. Bailliere Tindall/W.B. Saunders.

The well-defined pathological substrate is glomerulosclerosis, which can be induced in laboratory animals after 5/6 nephrectomy and subsequently by feeding the animals with a high protein diet. DPR in these animals on the other hand, leads to decreased single-nephron glomerular filtration rate (SNGFR) and less glomerulosclerosis, followed by a better survival[1]. Proteinuria decreases, like in humans[52].

ad 2) Recent studies suggest an important role of what we now call 'compensatory renal growth'[122]. After reduction of the functional renal mass, the glomeruli increase in size under the influence of still unknown growth factors. The podocytes, however, resting with their junctions on the glomerular basement membrane are not able to enlarge or to increase their number. Through the pores, thus created between the foot processes, proteins, lipids, as well as macrophages pass and cumulate in the mesangial area, thus contributing to the development of glomerulosclerosis. Maybe DPR could exert its effects not only by means of reducing intraglomerular pressure, but also by an 'anti-proliferative effect' as could be shown for the ACE-inhibitors[131].

ad 3) Many renal diseases are immunologically-mediated. Glomerulonephritis and certain types of interstitial nephritis at a tubular level are here the best examples. At the tubulointerstitial level often infiltrates can be seen long after healing of the primary attack. Agus et al[123] showed in 1985 that DPR modifies the progression rate in a model of anti-tubular basement membrane antibody-induced interstitial nephritis. Despite continued persistence of high antibody titres the immune-mediated interstitial injury was diminished. It was speculated that this could be due to the effect of DPR on T-cell mediated injury.
Furthermore, a common finding in renal disease is deposition of complement factor C_3 around renal tubules. Whereas in the 5/6 nephrectomy model there is an increase in tubular function, particularly in ammonia production, it is hypothesized that the increased intrarenal ammonia could trigger the alternative pathway of complement formation[124]. Then, amidated C_3 could act as a convertase, generating chemotaxis and the membrane attack complex which together with the leucocyte C_{3b} receptor could lead to inflammation. Reducing dietary protein results in reduced acid load and consequently lower production of ammonia in remnant nephrons. This is supported by the fact that Nath[125] et al could prove that chronic sodium bicarbonate loading reduces renal injury and peritubular deposition of complement.

ad 4) Seney and Wright[126] showed that variations in GFR after changing dietary protein intake at least partially depend on the tubuloglomerular feedback (TGF) system. They stimulated GFR by giving Sprague-Dawley rats 40% protein containing diets, and compared them with those on a 6% diet. By means of measuring SNGFR they found that the TGF response in rats fed the low protein diet was half-maximal at flows of 14-15 nl/min. In contrast, in animals fed the high protein diet it was half-maximal at 22-24 nl/min. Their conclusion was that the sensing mechanism on the TGF system is rendered less responsive by a high protein diet and that this change permits GFR to increase. Long-term stimulation of GFR may lead of the above mentioned damage to the glomerular basement membrane.

ad 5) An increased calcium-phosphate product is a well-known risk factor for the kidney. Diets, poor in protein content are also phosphate restricted. Maschio[83] has shown that protein most likely plays a more important role than phosphate in the pathogenesis of progressive renal insufficiency. In contrast, Lumlertgul et al[127] showed that phosphate restriction, independent of the protein intake arrests the progression rate. There is no doubt about the deleterious effects of a too high phosphate level. An important advantage of a low-protein diet is, that patients also need significantly less phosphate binders. This again reduces the risk of aluminium intoxication.

ad 6) Often a diet low in protein contains less lipids. In a review article Klahr[128] discussed the literature on manipulation of dietary lipid intake on chronic renal failure. Lipids may interfere with renal function through several mediators including the prostaglandin (PG) system. PGE_2 and Prostacyclin (PGI_2) are well-known potent glomerular vasodilatators and increase GFR[140,141]. Firstly, by effects that directly affect renal hemodynamics. During long-term high protein intake increased urinary excretion as well as glomerular production of PGE and 6-keto $PGF_{1\text{-alpha}}$ was noted[142-144]. Several studies reported blunting of the hyperfiltration response in man after pretreatment with a cyclo-oxygenase inhibitor[145,146], whereas others could not block the meat meal-induced GFR increase by means of indomethacin[1-7], or were only able to do this in patients with lupus erythematosus[148]. Secondly, by effects on the coagulation system and the platelet-endothelial cell interaction. It is speculated that substances like aspirin, dipyridamole, thromboxane synthetase inhibitors and heparin influence renal progression in a positive way. The suspicion rose, that certain PG, like thromboxane, enhance the platelet-dependent capillary thrombosis in the glomerular tuft and thereby induce progression.

Recommendations in using the diet

Based on the here presented data from clinical trials and supported by the theoretical backgrounds, the following guidelines for use of the diet in daily practice can be recommended with caution and awaiting the results of other trials.

1) Since trials up to now show only benefit in patients with glomerular disorders, the diet should be applied solely in patients with primary glomerular disease.

2) For the other diagnosis groups, especially for patients with early stages of diabetic nephropathy, a 'normalisation' of the diet should be advocated. In most cases this means 0.8 g/kg of protein per day.

3) In patients with proteinuria at least an attempt to reduce protein-excretion by means of 0.5 g protein/kg per day should be undertaken, even despite a (near) normal clearance.

4) If the diet has not the desired effect on progression and/or proteinuria, the addition of a low-dosed ACE inhibitor, even in case of normal systemic blood pressure, should be encouraged.

5) To maintain patient compliance during dietary treatment the frequent follow-up by an engaged dietician is mandatory, discussing all available data with the patient[136]. The 24 hour urea excretion can be used as a rough estimate to monitor protein intake. The formula to calculate protein intake in grams per day was in the 'Groningen Trial':

3 x urea excretion (in grams/24 h) +15, or
0.18 x urea excretion (in mmol/24h) +15.
However, recent information learns that this formula overestimates the protein intake and a very useful alternative was published in 1985 by Maroni et al[132], which also takes into account body weight and urinary protein loss. The 'Maroni formula' for calculating the daily protein intake in grams is:
6.25 x (0.46 x urea excretion (grams/24h) + 0.031 x kg body weight) + urinary protein excretion (in grams/24h).

6) For patients with advanced renal failure (GFR 10-20 ml/min) or with uremia, the additional administration of essential amino-acids, or better their keto-analogues, is necessary if the diet contains less than 0.5 g/kg of protein per day.

7) DPR is an effective method to lower serum phosphate, thus making patients less at risk regarding aluminium intoxication.

Bearing these rules in mind, protein-restricted diets can be an excellent tool in the hand of the nephrologist to prolong the patients' pre-dialysis life period in a very satisfying and safe way.

The obscure backgrounds of the working mechanism, which forced El Nahas and Coles to pose their ten questions[18], subsequently answered by Giovannetti[96], should not discourage the clinician to use protein restriction in daily practice.

Appendix

Letters to the editor in response to Chapter 2

In this appendix the three 'Letters to the Editor' that appeared in The Lancet (Lancet I; 102, 1985 and Lancet I; 465, 1985), as response to our article on the two' years follow-up of the patients (Chapter 2 of this thesis) are reproduced. Our answer to the letters a) and b) was published in the Lancet and can be found in chapter 2b. To the third Letter by Bock and Brunner we could not take position since their questions were published in the same issue as our answer to the other letters. The main topics however, are resolved in the letter of chapter 2b and in the consecutively published articles.

a)

SIR, We read with great interest the article by Dr Rosman and colleagues[52] on the effects of dietary protein restriction upon the progression of chronic renal failure. Their preliminary results, indicating that patients with chronic glomerulonephritis show a better response to dietary protein restriction than do patients with polycystic renal disease or nephrosclerosis, accords with our observations.

We did a retrospective analysis of 47 patients with chronic renal failure who were prescribed a protein-restricted diet (0.6 g/kg body weight daily) over a mean period of 17.4 months (±2.2 months SEM). The values for the reciprocal creatinine slopes for patients with chronic glomerulonephritis improved, as did those for patients with chronic pyelonephritis. However, patients with polycystic renal disease showed a blunted response (Table).

Reciprocal creatinine slopes

	Slope (x 10^4)		
Group	before	after	p
Chronic glomerulonephritis (n=10)	-1.53±0.33	-0.65±0.19	<0.01
Chronic pyelonephritis (n=13)	-0.50±0.07	-0.24±0.05	<0.01
Polycystic renal disease (n=5)	-0.94±0.25	-0.74±0.27	NS

We agree with Rosman et al that further evaluation is required to detect patients who will most benefit from dietary protein restriction, and to identify the point in the decline in their renal function at which protein restriction should be instituted.

Williams AJ, Bennett SE, Walls J.
Leicester, United Kingdom.

b)

SIR, Dr Rosman and colleagues conclude that 'moderate protein restriction is an acceptable and effective way of delaying functional renal deterioration' in chronic renal failure. This conclusions is based solely on the slower increase of serum creatinine concentrations in the groups of patients on a protein-restricted diet than in the control groups.

The reliability of serum creatinine levels as an index of glomerular filtration rate (GFR) when the diet is altered has never been adequately tested. The quantity and quality of food intake influence creatinine excretion[134,135], and thus the serum creatinine level, if the GFR is unchanged. In Rosman's study serum creatinine concentrations fell when the protein-reduced diets were started, from time 0 to 3 months. From 3 months onward the slopes of the curves for inverse creatinine for the control groups appear to be almost identical (fig 3 of chapter 2[a]). In group C, on a protein-restricted diet, urinary creatinine excretion decreased significantly with time (table V of chapter 2[a]), rendering the serum creatinine values, inverted or not, less useful indicators of GFR. It is not satisfactory to claim, without supporting statistical analysis, that the magnitude of certain of the observed differences between the groups are not explained by a decreased creatinine excretion, as Rosman et al do in their discussion.

Creatinine excretion and serum creatinine were measured every 3 months so creatinine clearance could have been calculated. Creatinine clearance is not a perfect measure of GFR, but it is probably adequate for the questions asked in this study. Was there a significant difference in the pattern of creatinine clearance between the diet groups and the control groups? If not, this carefully planned and executed study shows that the ability of a low-protein diet to retard the progression of renal insufficiency is very small or even non-existent.

Westberg G,
Göteborg, Sweden.

c)

SIR, We would like to support Dr. Westberg's criticism (see above) of Dr. Rosman and colleagues' paper[52]. In contrast to Rosman et al, who conclude that a low-protein diet

slows the progression of renal failure, we believe that this large, well-designed study demonstrates no relevant benefit from protein restriction.

Rosman's conclusions were based on creatinine levels, but serum creatinine is an inadequate index of glomerular filtration rate, unless creatinine excretion is constant, which it was not. Moreover, it seems odd to construct 'survival curves' with 'death' equated with a 10% increase in serum creatinine (fig 2 of Rosman et al).

We have used their data on urinary creatinine excretion together with the regression equations for 1000/creatinine to compute mean creatinine clearances (table).

Creatinine clearance of four study groups versus time

Group		Creatinine clearance (ml/min) at:		
		0 mo	9 mo	18 mo
A1	(unrestricted diet)	43.0	39.1	43.6
A2	(unrestricted diet)	16.2	13.7	11.4
B	(0.6 g protein/kg/day)	39.3	38.8	39.1
C	(0.4 g protein/kg/day)	19.4	17.7	15.7

The patients with moderate renal failure (groups A1 and B) did not benefit in any way from protein restriction; their creatinine clearance remained essentially constant over the 18 month observation period. In contrast, both patient groups with more advanced renal failure (group A2 and C) showed a progressive loss of creatinine clearance, which was only slightly greater in the protein-restricted groups (3.7 vs 4.8 ml/min). Hence, the differences in the evolution of serum creatinine may largely be accounted for by differences in creatinine excretion, which increased in group A1, remained stable in groups B and A2, and decreased in group C. We doubt that Rosman and colleagues' explanation that the decrease in creatinine excretion in the protein-restricted group C was merely due to diminished intake of meat because the steady downward trend, present at 3 months, continued at 9 and 18 months. More probably this represents decreasing endogenous creatinine production caused by progressive loss of muscle mass. This study seems to confirm F. Parsons' saying that 'all a low protein diet does is to shrink the patient down to the size of his kidneys'.

Bock HA, Brunner FP,
Basel, Switzerland.

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Summary

In recent years growing insight has been gained into the relationship between nutrition and certain patho-physiological conditions.

Since a long time we know that the typical diet in the Western World contains too much calories, fat and proteins. Thus, the World Health Organisation has several times changed its strategies and almost everytime the advice was given to reduce the intake of these nutrient components.

This thesis focuses on the relationship between dietary protein intake and the progression of renal disease.

Most kidney diseases show an inevitable progression towards end-stage renal disease, where the patient ultimately becomes dialysis-dependent or needs a transplantation to survive. The underlying mechanisms of this progression are still under discussion, but a major factor involved is an increased pressure in the glomerular tuft of remnant nephrons once renal mass is reduced. In an attempt to correct functional loss the remnant nephrons are hyperperfused, start hyperfiltering (increased single nephron glomerular filtration rate=SNGFR) and progress to glomerulosclerosis, initiated by leakage of proteins and macromolecules across the glomerular capillary wall and the accumulation of macrophages into the mesangium.

From animal experiments it is known that reducing protein intake in rats after 5/6 nephrectomy reduces SNGFR and prolongs survival. Retrospective studies in man confirm such a beneficial effect of dietary protein restriction on the progression rate of renal failure.

Definitive proof, however, can only come from a large, prospective trial with randomized patient allocation, and to meet this challenging concept is the subject of this thesis.

The results obtained after two years of follow-up in 228 patients are presented in *chapter 2*. This prompted to general optimism in applying the protein restricted diet. Using several statistical modalities, a beneficial effect of the diet in our nephrologic population was proven. Furthermore, the diet tended to decrease protein excretion and patients on a protein-restricted diet needed less phosphate binders to control their serum phosphate levels. However, clustering patients into certain diagnosis groups, the benefit regarding progression rate was limited to a few diagnosis groups, namely patients with various forms of glomerulonephritis. This is the subject of *chapter 3*. Still, there remained a statistical problem since the follow-up time at that moment was only two years, and such a period may be too short to draw unambiguous conclusions. Another methodological criticism to our study as presented in chapter 2, the use of the reciprocal serum creatinine values over time, is taken away in chapter 3, since we now used creatinine clearances. It must be sta-

ted that the ultimate results did not differ from those obtained with the reciprocal serum creatinine values.

To further clarify methodological problems encountered in large scale trials where an intervention in patients with progressive renal failure takes place, *chapter 4* discusses the relation between serum creatinine value and the creatinine clearance. This relationship has a poor correlation coeficient and is also strongly dependent on the degree of renal failure and the dietary advice. So, the method advocated by Mitch and Walser in 1976 to plot the reciprocal serum creatinine values over time, a routine follow-up procedure for many nephrologists, should be discouraged.

In *chapter 5* we related the initial protein excretion to the chance of success with dietary manipulation and concluded that patients with low initial proteinuria have the best outlook.

Chapter 6 presents the results obtained after four years of follow-up and gives a new dimension to the ideas of protein- restricted diets. After four years of follow-up, beneficial effects of the diet could only be proven in patients with glomerulonephritis and especially so if they were male. There was no evident general beneficial effect in contrast to the two years' follow-up. All patients that were slowly progressing after two years of diet appeared to be in line with patients on a free diet after four years. One can conclude that the usefulness of dietary treatment is temporary. Since urea excretion in our patients remained constant, it is not a compliance problem.

Chapter 7 discusses a major criticism to the diet, namely that it causes malnutrition. We conclude that there does not yet exist a reliable parameter to measure nutritional status objectively. An attempt is made to use amino-acid (AA) profiles, as well as their keto-analogues to asses the state of nutrition. All patients showed the typical AA-pattern of uraemia. No differences, however, were seen between protein-restricted and free-diet patients. So, if the amino-acid profile is a parameter to diagnose malnutritive states, protein restricted diets do not cause a protein-malnutrition.

Chapter 8 adresses the influence of gender on the progression rate of chronic renal failure. As in the animal experiment, male patients showed a more rapid decline towards end-stage renal failure. On the other hand, they also responded better to the diet whereas female patients showed no response at all. The underlying mechanisms, mainly based on data derived from animal experiments, are discussed.

Summarizing: The protein-restricted diet deserves a place in the treatment of patients with chronic renal failure. Especially in male patients with chronic glomerulonephritis, already early in the course of the disease a diet containing 0.5 g protein/kg BW/day should be applied, if possible mainly from vegetable origin. When clearance values of about 30 ml/min are reached, the diet can be further restricted; in this case keto-analogue amino acids should be supplemented. Now that the diet is becoming increasingly important, countries like The Netherlands, where keto acids are not available , should consider registration of this vital supplement.

We did not find any sign of malnutrition in our patients over a four years' period. It should, however, be remarked that still no good parameter to assess nutritional status is available.

Furthermore, protein-restricted diets have the advantage of reducing proteinuria in most patients and the need for phosphate binders is reduced, thus lowering the risk of aluminium-related complications.

To further define the right target groups and maybe clarify the theoretical backgrounds of this important therapeutic modality, we have to await large multicenter trials as they are running at the moment in Europe and in the United States.

Samenvatting

De laatste jaren is er een toenemend inzicht ontstaan in de samenhang tussen voeding en patho-fysiologische condities. Zo is reeds langere tijd bekend dat voeding in de Westerse wereld een enorm surplus aan calorieën, vet en eiwit bevat. De Wereldgezonheidsorganisatie (WHO) optimaliseert regelmatig de normen en bijna steeds adviseerde zij tot verdere reductie van de inname van deze voedingsbestanddelen.

Dit proefschrift bespreekt de relatie tussen de diëtetische eiwittoevoer en de progressie van chronische nierziekten.

Nieraandoeningen hebben van nature de neiging, om, wanneer ze eenmaal bestaan, langzaam voort te schrijden in de richting van de terminale nierinsufficiëntie, waarna de patiënt dialyse-behoeftig wordt. Hoe dit proces exact verloopt, is nog onbekend maar men vermoedt dat bij afname van het aantal functionerende nefronen de nierfunctie in eerste instantie behouden blijft door toegenomen perfusie en filtratie van de resterende nefronen (toename van de 'single nephron glomerular filtration rate': SNGFR) en dat door de aldus geïnduceerde verhoogde intracapillaire druk de glomerulaire basaalmembraan te zeer belast wordt. Er treedt lekkage van eiwit, grootmoleculaire stoffen en macrofagen naar het mesangium op en het eindproduct van de aldus ontstane vicieuze cirkel is glomerulosclerose.

In het remnant kidney-diermodel (5/6 nephrectomie bij de rat) was reeds bekend dat reductie van de eiwitinname de SNGFR doet afnemen en de overleving verbeterd. In mensen kon retrospectief worden aangetoond dat eiwitbeperkte voeding een vertragend effect op nierfunctieachteruitgang heeft.

Voor de definitieve bewijsvoering is echter een prospectieve, gerandomiseerde studie in een groot aantal patiënten nodig en een dergelijke studie vormt de basis van deze dissertatie.

Tweehonderdzevenenveertig patiënten werden gerandomiseerd ingedeeld in een eiwitbeperkte groep (0.4-0.6 gram eiwit per kg. lichaamsgewicht per dag) of in een controlegroep, die het normale dieet continueerde. De resultaten, met name wat betreft de nierfunctie, zoals die na twee jaar 'follow-up' van de aldus ingedeelde groepen werd verkregen, worden besproken in *hoofdstuk 2*. Deze publicatie vond in 1984 plaats en de resultaten waren, over de hele patiëntenpopulatie gerekend, positief. Gebruik makend van verschillende statistische methodieken kon een vertragend effect van het dieet op de progressie van de nierinsufficiëntie worden aangetoond. Bovendien leidde toepassing van het dieet tot een afname van de proteinurie en patiënten op een eiwitarm dieet hadden minder fosfaatbinders nodig.

In *hoofdstuk 3* wordt nader ingegaan op het effect van het dieet in de verscheidene diagnosegroepen, en het blijkt dat patiënten met glomerulonefritis het meest van het dieet profiteren. In dit hoofdstuk wordt tevens op een der kritieken die op de eerste fase van de studie werd uitgeoefend ingegaan, namelijk het gebruiken van de reciproke serum creatininewaarden tegen de tijd. De berekeningen werden nu uitgevoerd met de creatinineklaring. Hierbij moet opgemerkt worden dat dit de uitkomst niet veranderde.

In *hoofdstuk 4* wordt op dit gegeven nog nader ingegaan en het verband tussen serum creatinine waarden en de creatinine klaring uitvoerig geanalyseerd. Deze relatie blijkt sterk afhankelijk van de mate van nierinsufficiëntie en of er dieetinterventie plaats vond. Geconcludeerd wordt, dat de methode, reciproke serumcreatininewaarden te plotten tegen de tijd, niet aanbevelenswaardig is.

In *hoofdstuk 5* correleerden we de initiële eiwituitscheiding met het therapiesucces van de dieetmanipulatie en concluderen dat patiënten met een lage eiwitexcretie bij studieaanvang de beste vooruitzichten hebben. Verder reduceert eiwitbeperking de proteinurie significant.

Hoofdstuk 6 bespreekt de resultaten zoals die na 4 jaar 'follow-up' werden verkregen, en hier blijkt in versterkte mate dat het nagenoeg uitsluitend de patiënten met glomerulonefritis zijn die op het dieet aanspreken. Dit geldt nog in versterkte mate indien ze van het mannelijk geslacht zijn. Voor vrouwen kon geen significante vertraging door het dieet worden aangetoond. Over de gehele groep gerekend bracht eiwitbeperkte voeding geen verbetering meer teweeg, dit in tegenstelling tot de resultaten zoals die na 2 jaar 'follow-up' waren verkregen. Men zou hieruit kunnen concluderen dat het therapiesucces maar tijdelijk is. In ieder geval wordt het onderscheid niet veroorzaakt door een afname van de dieetcompliantie, want de ureumuitscheiding bleef constant.

Hoofdstuk 7 bespreekt een algemene kritiek op eiwitbeperkte voeding, namelijk dat ze tot eiwit-ondervoeding zou leiden. In de eerste plaats moet geconcludeerd worden dat er nog steeds geen betrouwbare parameter is om de voedingstoestand te objectiveren. In deze studie wordt een aanzet gedaan de aminozuren-profielen, met name die van de keto-analoge vormen, hiertoe te gebruiken. Al onze patiënten vertoonden de typische aminozuren-profielen der uraemie. Er werd geen verschil geconstateerd tussen eiwitbeperkte- en controlegroep patiënten. Indien de aminozuren-profielen bruikbaar zijn als maatstaf voor de voedingstoestand, is er bij onze eiwitbeperkte diëten dus geen sprake van eiwit-ondervoeding.

Hoofdstuk 8 gaat dieper in op de invloed van het geslacht op de progressie van nierinsufficiëntie. Evenals in het dierexperiment, blijken mannen een snellere progressie in de richting van terminale nierinsufficiëntie te vertonen. Aan de andere kant waren het echter ook juist de mannelijke patiënten die profiteren van het eiwitbeperkte dieet. In dit hoofdstuk worden de theoretische achtergronden hiervan besproken.

Samengevat valt te zeggen:

Eiwitbeperkte diëten verdienen een plaats in de behandeling van patiënten met nierziekten. Vooral bij mannen met glomerulonefritis moet al vroeg in het verloop, als de functie nauwelijks zichtbaar beperkt is, begonnen worden met een dieet dat 0.5 g/kg/dag aan eiwit bevat, het liefst van plantaardige oorsprong. Indien tot een sterkere reductie van de eiwitinname moet worden overgegaan, dienen keto-analoge aminozuurpreparaten te worden gesuppleerd. In dit verband is erop te wijzen dat deze preparaten in Nederland helaas nog niet zijn toegelaten, dit in tegenstelling tot de rest van Europa.

Eiwitbeperkte diëten leiden, in tegenstelling tot wat vaak beweerd wordt, niet tot ondervoeding.

Om de exacte doelgroep te kunnen omschrijven is het wachten nog op de resultaten van grotere, multicenter studies, die op dit moment in Europa en in de USA plaatsvinden.

Overzicht voor de geïnteresseerde leek

De laatste jaren is er een toenemend inzicht ontstaan in de samenhang tussen voedingpatronen en de gezondheid. Zo is reeds geruime tijd bekend dat onze voeding in de Westerse wereld een enorm overschot aan calorieën, vet en eiwit bevat. De Wereldgezondheidsorganisatie (WHO) stelt met regelmatige tussenpozen normen voor wat betreft de normale voedingsgewoonheden vast en bij deze gelegenheden adviseerde zij steeds verdere reductie van deze voedingsbestanddelen.

Dit proefschrift bespreekt de relatie die wij veronderstellen tussen de eiwitinname met de voeding en het voortschrijden (progressie) van nieraandoeningen. Nierziekten hebben van nature de neiging, om, wanneer ze eenmaal bestaan, langzaam voort te schrijden in de richting van de zogenaamde terminale nierinsufficiëntie, waarna de patiënt aan de kunstnier moet. Waarom nierziekten spontaan verslechteren hoewel de oorspronkelijke oorzaak al is behandeld weten we nog niet precies. Elk van onze beide nieren bevat circa 1 miljoen kleine eenheden, nefronen genaamd, waarin een minuscule vaatkluwen ligt die een doorlaatbare wand heeft voor water en voor de giftige stoffen die zich ophopen in het bloed. Zo heeft een normale volwassene 2 miljoen van deze 'filtertjes': glomeruli genaamd. Indien door de een of andere oorzaak het totale aantal van deze glomeruli gereduceerd wordt moeten de overige al het werk doen en dit leidt tot een overbelasting. In eerste instantie zullen de glomeruli dit nog weten op te vangen, maar na een zekere tijd is de reserve verbruikt en zullen meer en meer glomeruli het begeven (vicieuze cirkel) doordat de druk op de membraan die alleen water en gifstoffen door moet laten te groot wordt. Het gevolg is, dat de zeer kleine poriën in de membraan zich vergroten en eiwit zullen doorlaten, hetgeen dan in de urine gevonden wordt: vaak het eerste teken dat er met de nier iets niet in orde is.

Uit dierexperimentele modellen was reeds langere tijd bekend dat vermindering van de dagelijkse eiwitinname de druk in genoemde glomeruli verlaagt en de daaruit ontstane theorie dat eiwitreductie met de voeding de progressie van nierziekten (in gunstige zin) beïnvloedt, moest daarom bij mensen worden getoetst.

Vanaf ongeveer 1980 wordt er in deze richting onderzoek gedaan. Giovannetti, Giordano en Maschio, (leiders van Italiaanse onderzoeks-werkgroepen) onderzochten retrospectief, d.w.z. over de daaraan voorafgaande jaren, hun patiënten op de relatie tussen eiwitinname en progressie van de nierinsufficiëntie. Ze vonden allen dat een lagere eiwittoevoer met de voeding de patiënt minder snel naar het stadium van terminale nierinsufficiëntie leidt.

Het uiteindelijke bewijs kan echter alleen geleverd worden als er aan minstens 3 wetenschappelijke voorwaarden is voldaan, te weten:

a) het onderzoek moet *prospectief* zijn, d.w.z. de patiënten moeten worden opgenomen in een studie en dan in de loop der tijd intensief vervolgd,

b) er moet een *controlegroep* zijn die geen dieetbehandeling ondergaat; deze controlegroep moet precies zo samengesteld zijn als de behandelingsgroep,

c) het onderzoek moet *gerandomiseerd* starten, d.w.z. patiënt en arts hebben geen vrije keuze of de patiënt in de controle-, dan wel in de behandelingsgroep terecht komt. Er dreigt anders het gevaar van 'selectionering', d.w.z. de meest gemotiveerde patiënten zullen voor eiwitbeperking kiezen en het resultaat zal dan eerder positief zijn.

Onze studie is internationaal de eerste in zijn soort die aan al deze voorwaarden voldoet.

De resultaten, zoals die na twee jaar 'follow-up' werden verkregen, worden besproken in *hoofdstuk 2*. Deze publicatie vond in 1984 plaats en de resultaten waren, over de hele patiëntenpopulatie gerekend positief, d.w.z. eiwitarme voeding gaf een vertragend effect op de progressie van het nierfunctieverlies te zien.

Een verdere uitbreiding van onze gegevens presenteren we in *hoofdstuk 3*. Met name worden hier de verschillende groepen nieraandoeningen separaat beschouwd. Het blijkt dat niet voor elke nierpatiënt eiwitarme voeding de ideale behandeling is. Een probleem was echter dat we op het moment van schrijven van het artikel uit moesten gaan van een follow-up duur van twee jaar, mogelijk nog te kort om een betrouwbare uitspraak te doen. *Hoofdstuk 4* beschrijft een aantal problemen, zoals we die bij de statistiek van dit soort studies tegenkomen. Zo blijkt dat de interpretatie van gegevens, zoals we die uit retrospectieve studies verkrijgen, zeer sterk afhankelijk is van de statistische methode die gebruikt wordt. Dit geldt dit helaas ook voor prospectieve studies, zij het in mindere mate.

De relatie tussen de eiwitinname en de vaak met nierziekten gepaard gaande verhoogde eiwituitscheiding met de urine, wordt besproken in *hoofdstuk 5*. Eiwitbeperking blijkt tot een belangrijke afname van het eiwitverlies met de urine te leiden: passend in de theorie dat eiwitbeperking leidt tot een afname van de druk in de glomeruli. Verder blijken patienten met geringe eiwituitscheiding bij studieaanvang de meeste kans op succes met het dieet te hebben.

In hoofdstuk 6, moet een deel van de optimistische verwachtingen van het dieet, zoals die uitgesproken werden na 2 jaar follow-up in 1984 (zie hoofstuk 2), weer teruggenomen worden. Het blijkt dat na 4 jaar eiwitarme voeding een speciale sub-groep van de patiënten het meest van het dieet profiteert en wel de patiënten met glomerulonefritis (een soort chronische, niet door een bacterie of een virus veroorzaakte ontsteking van de nieren), vooral als de patiënten van het mannelijk geslacht zijn.

Veelgehoorde kritiek voor wat betreft de eiwitarme voeding, is dat deze een ondervoedingstoestand veroorzaakt, die tot eiwittekort leidt. Hierbij doet zich het probleem voor dat we geen betrouwbare parameters hebben om de voedingstoestand vast te leggen. De spiermassa laat zich moeilijk in getal uitdrukken en ook laboratoriumgegevens zijn niet in alle studies betrouwbaar gebleken. In een poging hier een nieuw concept te introduceren bepaalden we bij 109 van onze patiënten een zeer uitvoerig aminozurenprofiel in het bloed en de resultaten daarvan met mogelijke gevolgen voor de beoordeling van de voe-

dingstoestand worden besproken in *hoofdstuk 7*. Het is mogelijk dat de zogenaamde ketoanalogen van bepaalde aminozuren een goede parameter zijn om te bepalen of de patiënt zich (nog) in een goede voedingstoestand bevindt. In onze patiënten stelden we vast dat de aminozurenprofielen het typische beeld van de nierinsufficiëntie weerspiegelen. In ieder geval vertoonden de patiënten op eiwitarme voeding geen ander aminozurenprofiel dan die, welke hun gebruikelijke dieet voortzetten. Omdat we ook bij andere onderzoeken geen aanwijzingen vonden dat de eiwitbeperking de voedingstoestand ongunstig beïnvloedt, nemen we aan dat de soms toch forse reductie van de eiwittoevoer geen ondervoedingstoestand veroorzaakt.

Tot slot wordt in *hoofdstuk 8* de invloed van het geslacht op de snelheid van achteruitgang van nierfunktie besproken. Uit dierexperimenten bleek recentelijk dat mannelijke ratten sneller in het stadium van terminale nierinsufficiëntie komen dan vrouwelijke ratten. Voordien was dit niet opgevallen, daar in het algemeen mannelijke ratten gebruikt worden voor dierexperimenten. Het gegeven, dat mannelijke patiënten een snellere spontane progressie hebben dan vrouwelijke, konden wij in onze patiënten bevestigen, met daarbij de klinisch relevante bevinding dat het tevens de mannelijke patiënten zijn die het meest profiteren van het dieet.

Met dit, onze doelgroep scherp omschrijvende hoofdstuk wordt het proefschrift afgesloten.